University of Alberta

Shiga toxins Binding to P^k Trisaccharide Analogs

by

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Abstract

We have synthesized and investigated the P^k-trisaccharide and its deoxy analogs as inhibitors of cell surface adhesion processes. The common α-Gal*p*-(1→4)-β-Gal*p* epitope of the natural globoseries saccharides is the receptor site of the Shiga toxins. We conducted chemical mapping studies of P^k trisaccharide-Shiga toxin Type 1 interactions using 7 deoxy analogs of P^k-trisaccharide. Synthesis of the 2′, 3′, 6′, 2″, 3″, 4″ and 6″-monodeoxy analogs of methyl globotrioside was performed using a variety of deoxygenation methods.

FTICR-MS was used to evaluate the binding affinity of the P^k-trisaccharide and its deoxy analogs. The 3″-deoxy trisaccharide **24** showed the lowest affinity of all ligands tested. Surprisingly, the 6″-deoxy trisaccharide **26** was found to exhibit similar binding affinity to the native ligand despite a crystallographically documented involvement of 6″-OH in hydrogen bonding with the protein in all 3 putative binding sites.

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To the glory of God. The future is bright if Christ is your hope.

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Abbreviations

AC	acetyl
AIBN	2,2'-azobisisobutyronitrile
Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Bn	benzyl
Bz	benzoyl
CSA	camphorsulfonic acid
DCM	dichloromethane
DMF	*N',N'*-dimethylformamide
ER	endoplasmic reticulum
ERC	endosomal perinuclear recycling compartment
ES	electrospray
Et	ethyl
FAC-MS	frontal affinity chromatography mass spectrometry
FTICR-MS	Fourier transform ion cyclotron resonance mass spectrometry
Gal	galactose

GalNAc	*N*-acetylgalactosamine, 2-acetamido-2-deoxy-D-galactose
Gb_3	globotriaosyl ceramide
Gb_4	globotetraosyl ceramide
Glc	glucose
Glu	glutamic acid
HC	hemorrhagic colitis
HUS	hemolytic uremic syndrome
ITC	isothermal titration microcalorimetry
Leu	leucine
LR	ligand receptor
Me	methyl
MS	molecular sieves
NIS	*N*-iodosuccinamide
Phe	phenylalanine
PMB	4-methoxybenzyl
Pry	pyridine
SE	2-(trimethylsilyl) ethyl
SPR	surface plasmon resonance
Stx	Shiga toxin
TfOH	trifluoromethanesulfonic acid
Thr	threonine
TLC	thin layer chromatography
TMS	trimethylsilyl

Tol	toluene
Trp	trytophan
UDP	uridine 5′-diphosphate

Chapter 1

Introduction

1.1 Role of carbohydrates and their interactions with proteins

Carbohydrates that facilitate interactions with organisms are on the surface of all living cells. Carbohydrates are important in the development, growth and function of biological systems, and are also significant as mediators of biomolecular recognition.

Cell surface carbohydrates act as "receptors" for viruses, bacteria, toxins, and other organisms (Figure 1.1). They exist mostly as carbohydrate conjugates including glycoproteins, proteoglycans, and glycolipids.[1] Carbohydrate–protein interactions through the interaction of cell surface molecules that contain an oligosaccharide and their receptors such as lectins are involved in many biological processes such as intercellular communication and differentiation.[2] The recognition site of a typical carbohydrate ligand bound by receptor proteins is often localized to a simple disaccharide or trisaccharide moiety of the oligosaccharides. The study of the recognition site of the receptor protein required the development of syntheses of natural and structurally modified oligosaccharides. These synthetic oligosaccharide epitopes are interesting and tangible target molecules, the development of which is an attractive task for novel therapeutics. Examples of such therapeutics that have already reached an advanced stage of development are drugs for influenza,[3] glycoconjugates used in cancer therapy,[4] and anti-HIV agents.[5, 6]

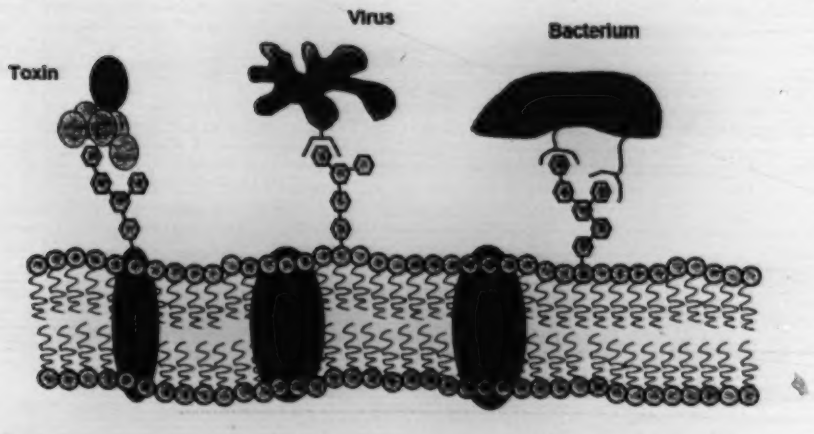


Figure 1.1 *Carbohydrate-Protein interactions on the cell surface*

1.2 Shiga Toxins: Principal virulence factor in bacterial disenteria

Dysentery is a disease that is characterized by bloody diarrhea.[7] It is classified in two types: amoebic dysentery, caused by *Entamoeba histolytica* discovered by Losch in 1875, and bacillary dysentery, which was discovered by Kiyoshi Shiga in 1898. Shiga toxin (Stx) is produced by *Shigella dysenteriae* and modern studies of Shiga toxin have been reported, which were carried out by the inoculation of Shiga toxin in ligated rabbit ileal loops in 1972.[8] While screening *Escherichia coli* using vero cells derived from African green monkey kidney, Konowalchuk found that some strains of *E. coli* had an irreversible cytotoxic activity.[7, 9] Verotoxin-producing *E. coli* strains have been shown to be associated with diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS).[10, 11] O'Brien et al. have shown that verotoxins have similar structure and biological activity to Shiga toxin (Stx), resulting in classification as Shiga-like toxins (SLTs).[12, 13]

Old nomenclature	New nomenclature	
	gen	Protein
Shiga toxin (Stx)	*stx*	Stx
Shiga-like toxin I (SLT-I) or Verotoxin 1 (VT 1)	stx_1	Stx1
SLT-II or VT2	stx_2	Stx2
SLT-IIc or VT2c	stx_{2c}	Stx2c
SLT-IIe or VT2e	stx_{2e}	Stx2e

Table 1.1 *Nomenclature of members of the Shiga toxin family* [14]

Two historical nomenclatures systems of Shiga toxins (sometimes designated as SLTs and in other occasions as VTs) have been used in the literature and cause confusion to novice researchers. The new nomenclature system for the Shiga toxin family was suggested by Calderwood et al. (Table 1.1), and this system is followed in this thesis.

1.2.1 Structure of Shiga Toxins

Shiga toxins belong to a family of protein toxins that have significant toxicity to a number of eukaryotic cells.[15] The members of the Shiga toxin family are bipartite molecules, composed of two parts: one part is responsible for binding to the cell surface and the other subunit for entering the cytosol of target cells and enzymatically inactivating protein synthesis.[16] In Figure 1.2 the carbohydrate binding moiety (B_5) and the enzymatically active moiety (A) are shown in a schematic model together with the crystal structure of Shiga toxin.

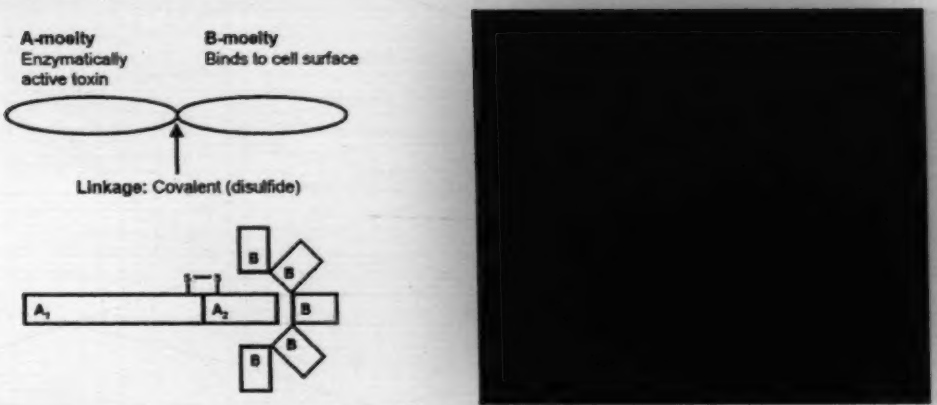


Figure 1.2 *Schematic model* [17] *and crystallographic structure of Shiga toxin (PDB protein data bank; 1DMO)*

The zymogen moiety (A) contains a loop formed by an internal disulfide bond. Proteolytic cleavage in the loop area and reduction of the disulfide linkage generates the A_1 and A_2 subunits. Subunit A_1 is the active enzyme, which promotes rapid cell intoxication.[18] The enzyme furin,[19] which is located in the Golgi apparatus and in endosomes initiates the processing of the Shiga toxin A moiety.[19] After the enzymatically active moiety A_1 is released in the cytosol it removes one adenine from the 28S RNA of the 60S ribosomal subunit thereby inhibiting protein synthesis.[20] The binding moiety (B_5) has a pentameric subunit that is responsible for binding of the toxin to specific glycolipid receptor on the surface of cells.[21] The weight of Shiga holotoxin is approximately 70 kDa, comprised of the 32 kDa catalytic A subunit and five copies of the 7.7 kDa B subunit.[22] The bacterial protease furin nicks the A subunit into a catalytically active 27 kDa N-terminal A_1 fragment and a 4 kDa C-terminal A_2 fragment.

The Shiga toxin family can be categorized into Shiga toxin (Stx) produced by *Shigella dysenteriae*, and Shiga-like toxin 1 (Stx1) and Shiga-like toxin 2 (Stx2) produced by *E. coli*.[22] The amino acid sequences of Stx and Stx1 differ only by a single amino acid in the A subunit, while the amino acid sequence homology between the A subunits and B subunits of Stx1 and Stx2 is only 56 %.[22, 23] The A subunit of Stx and Stx1 has 315 amino acids, while that of Stx2 has 318 amino acids.[16] The B subunits of all Shiga toxin family members have 89 amino acids. There are several variants of Stx2. Although all variants have a similar structure, they show different biological properties, immunological reactivity, or receptor specificity. Usually the Shiga toxin family binds preferentially to Gb_3 the glycolipid (α-D-Gal*p*-(1→4)- β-D-Gal*p*-(1→4)-D-Glc*p*-(1→O)ceramide,[16, 24, 25] except a variant, Stx2e that binds to Gb_4 (β-D-Gal*p*-NAc-(1→3)- α-D-Gal*p*-(1→4)- β-D-Gal*p*-(1→4)-D-Glc*p*- (1→O) Ceramide.[26]

1.2.2 Mechanism of Toxicity

First, Shiga toxins require a specific binding site on the responding cells. Most Shiga toxins bind to the glycolipid Gb_3 or Gb_4. The carbohydrate part of the glycolipid receptor is essential for the toxin-receptor interaction as well as the lipid tail of the receptor.[18, 22, 27] It is desirable to elucidate the mechanism of toxin action to provide a rational basis for treatment of infectious diseases, in which toxins are a pivotal pathogenic factor.[28, 29] Understanding toxin-receptor interaction at the molecular level provides valuable information for the treatment of infectious disease.

After Shiga toxin binds to the glycolipid Gb_3 at the cell surface, it is very efficiently endocytosed from clathrin-coated pits.[17, 25, 30, 31] Why Shiga toxin would aggregate in clathrin-coated pits is not yet elucidated. However, it was proposed that the extent of localization of Shiga toxin-receptor complex to lipid raft seems to be cell dependent.[32]

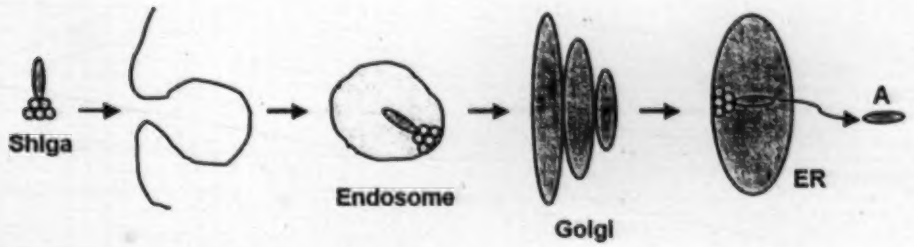


Figure 1.3 *Entry of Shiga toxin into cells* [24]

As shown in Figure 1.3, Shiga toxins in some cells are transported through the Golgi apparatus en route to the cytosol.[24, 33-37] Shiga toxins move from the endosome to Golgi apparatus via more than one pathway. One pathway, which is dependent on the small GTP-binding protein Rab9 transports mannose-6-phosphate receptors from late endosome to Golgi apparatus.[38-40] Transport of Shiga toxins to the Golgi apparatus,[24] the Golgi-associated protein TGN38,[34, 35] as well as B-subunit alone[33, 41] seems to occur through the endosomal perinuclear recycling compartment (ERC). However, more studies are necessary for the elucidation of the detailed transport pathway. Although the mechanism of Shiga toxin transport to the Golgi apparatus is not clearly understood, Shiga toxin B-subunit is a useful indicator to study the pH of both the Golgi apparatus and the endoplasmic reticulum (ER).[42]

After entry into the Golgi apparatus, the transport of Shiga toxin to the ER is shown to occur in a retrograde manner.[18, 43] A well known retrograde transport mechanism involves the KDEL receptor that is located in the Golgi apparatus.[44-46] This receptor induces retrograde transport by COPI-coated vesicles.[47] However, Shiga toxin lacks a KDEL sequence. This requires that the Shiga toxin family is transported by a different pathway from COPI-coated vesicles.[48-50] In fact, recent results indicate that the Stx1 B subunit is transported from the TGN to the ER by using a COPI-independent Rab6-dependent retrograde transport route.[51, 52]

The enzymatically active moiety (A) of the Shiga toxin has a loop structure between A_1 and A_2 subunit formed by an internal disulfide bond. Proteolytic cleavage of the loop is sensitively effected by furin in the TGN as well as in endosomes and promotes rapid intoxication. After cleavage by furin and reduction of the disulfide, the A_1 moiety is separated from the A_2 moiety in the ER. The release of the A_1 subunit into the cytosol inhibits protein synthesis and initiates apoptosis.

1.3 Shiga Toxin and Carbohydrate-Receptor Interaction

1.3.1 Thermodynamics of Binding Interaction

In aqueous solution, the association of ligand and receptor occurs when their cohesive power is larger than the total repulsive interaction between them. Non-covalent interaction between molecules is essential in living organisms and such bonds are substantially weaker than the energy of a covalent bond.[53] Although these interaction between carbohydrates and the binding sites of proteins are extremely weak, they are attractive and specific, and provide a dynamic framework for self-

regulation and adaptation to the system.[54, 55] The driving force of the binding process has multiple origins and a full understanding of the recognition process between carbohydrate and protein requires the measurement of several parameters and an ideally complete thermodynamic profile.[56, 57] The simple bimolecular association of ligand (L) and receptor (R) at equilibrium is shown in equation (1). At equilibrium, the system contains unbound L and R and the bound complex LR:

$$L + R \rightleftharpoons LR \qquad (1)$$

This equilibrium constant K_A is defined as:

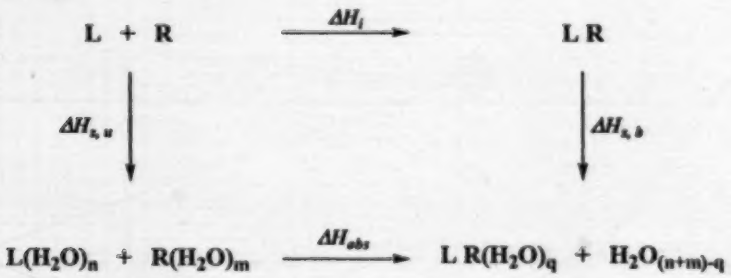
$$K_A = \frac{[LR]}{[L][R]} \qquad (2)$$

The association constant K_A and Gibbs free energy give some information about the strength of binding in the complex system, expressed in equation (3):

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_A = RT \ln K_D \qquad (3)$$

where R is the gas constant, T is the temperature (K), and free energy (ΔG), enthalpy-heat of binding (ΔH), and entropy (ΔS) are the thermodynamic parameters for the association of the LR complex. K_D is the dissociation constant ($1/ K_A$). These thermodynamic parameters provide information regarding the relative contributions of the different interactions (hydrogen bonding, van der Waals forces, etc.) that provide the driving force for formation of the LR complex system.

Since the differences in measured energy is a composite of structural change of the oligosaccharides or differential contribution, such as solvent effects,[58] an unambiguous deconvolution of the thermodynamic data is difficult. In aqueous solution,[59] both proteins and carbohydrates are generally hydrogen bonded to water. To associate with each other they have to break hydrogen bonds to water. Ligand-water and receptor-water hydrogen bonds are replaced by new ligand-receptor bonds. Accordingly, the overall binding energy is implied by the difference in energy between solute-solute versus solute-solvent interactions and new interactions of water with the complex (Scheme 1.1).



Scheme 1.1 *Born-Haber cycle showing separation of measured enthalpy of binding (ΔH_{obs}) into an intrinsic (Solute-Solute) enthalpy (ΔH_i) and enthalpies of solution for the bound ($\Delta H_{s,b}$) and unbound ($\Delta H_{s,w}$) systems* [60]

Studies of the thermodynamics of carbohydrate binding have shown entropy-enthalpy compensation.[61, 62] The entropic contribution to binding has been described as the sum of various contributions as shown in equation (4):[63]

$$\Delta S = \Delta S_{solv} + \Delta S_{config} + \Delta S_{rot} + \Delta S_{trans} \qquad (4)$$

As the interaction of ligand with protein become stronger (of higher enthalpy), this applies more constrains to the overall motion of atoms, therefore the entropy is increased and the corresponding entropy term for free energy becomes less favorable.

This enthalpy-entropy compensation has been interpreted both in terms of changes in rotational degrees of freedom and in terms of solvent reorganization.[64-66] Configurational and solvation entropies include free energy loss that occurs on binding.[67]

1.3.2 Structural Studies and Details of Interaction

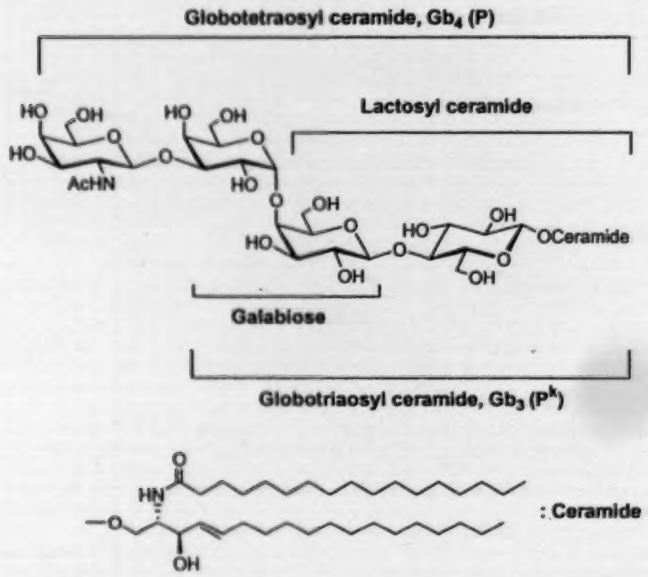


Figure 1.4 *The structure of P blood group antigen* [68]

The blood group system has a common P phenotype and rare P^k phenotype.[69] The structures of P and P^k antigens were elucidated as the glycosphingolipid globoside and trihexosyl ceramide (Figure 1.4). The ABO blood group antigens exist as glycolipids and glycoproteins, whereas the P blood group antigens only exist as glycolipids.[70] The P blood antigens are cancer antigens,[71] for example on Burkitt lymphoma cells,[72] human tetracarcinoma cells,[73] and human myeloid leukemia cells[74] and serve as receptors for bacteria and toxins, such as the Shiga toxin.[75]

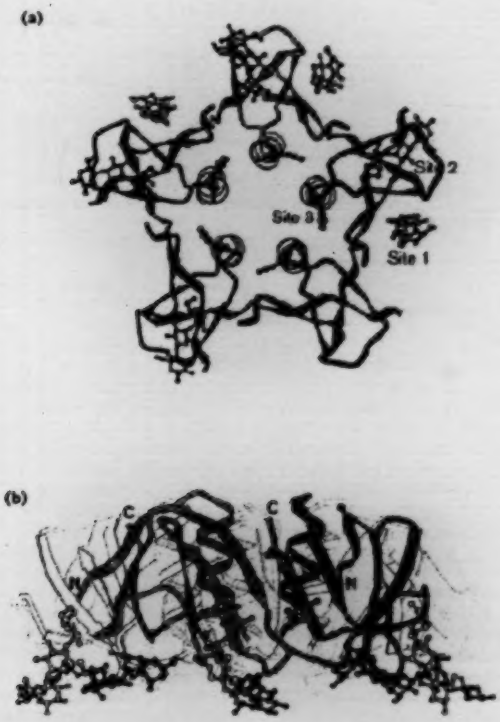


Figure 1.5 *Two orthogonal views of the GT3(Q65E/K67Q mutant oh Stx2e) B pentamer bound to the P^k-α-D-Galp-(1→4)-β- D-Galp-(1→4)-β- D-Glcp-(1→O) $(CH_2)_8CO_2CH_3$ trisaccharide glycoside*[76]

The pentameric B subunit of Shiga toxins recognizes glycolipid globotriaosyl ceramide (Gb_3) containing a galabiose moiety (α-D-Gal*p*-(1→4)-β-D-Gal*p*).[77] Globotriaosyl ceramide (P^k) is the major receptor for binding to the majority of the Shiga toxin family. However, globotetraosyl ceramide (Gb_4) is the preferred receptor for the pig pathogen Stx2e.[76] Although the exact role of the lipid is not fully understood, the binding of the toxin to the receptor changes with the nature of the lipid.[78]

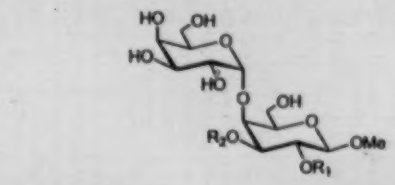
The crystal structure of the B pentamer complexed with the Gb_3 trisaccharide analog has been reported.[79] According to the crystal structure, the B pentamer has three binding sites per B subunit (Figure 1.5).

Sugar	Protein atom (Hydrogen bonds)	Protein residue (Hydrophobic Interaction)
Site 1		
αGal 4O	Thr 21	
5O	Thr 21	Leu 29
6O	Glu 28, Gly 60	
βGal 3O	Gly 60	Phe 30
6O	Asp 17	
Glc		Phe 30
Site 2		
αGal 2O	Asp 16	Phe 30, Thr 31
3O	Arg 33	Gly 62, Ser 64
4O	Asn 32, Arg 33	
6O	Asn 32, Phe 63	
βGal 6O	Asn 55	Thr 1, Thr 54
		Ala 56, Gly 62
Glc		Asn 55
Site 3		
αGal 4O	Asp 18	
5O	Trp 34	Trp 34
6O	Trp 34, Asn 35	
βGal		Trp 34

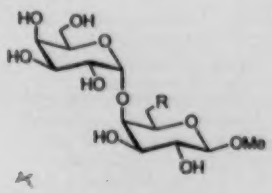
Table 1.2 *Hydrogen bonds and hydrophobic interaction between the sugar and the protein*

In site 1 which is formed by adjacent *B subunits, two hexose residues* of the carbohydrate ligand make hydrophobic contacts with Phe30 and Leu29, and hydrogen bonds with Asp17, Thr21, Glu28 and Gly60 (Table 1.2). Three sugar residues are engaged with site 2, which is formed by amino acid residues that belong to each individual B subunit. The ligand is almost equally involved in hydrogen bonds and hydrophobic contacts in both site 1 and site 2. In contrast, Gb_3 is bound to site 3 orthogonal to the B subunit surface, therefore site 3 has fewer hydrogen bonds than binding sites 1 and 2 and essentially contacts only the terminal α-Gal residue.

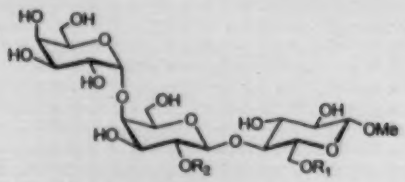
1.3.3 Design of Uni-and Multivalent antagonists



1 $R_1 = R_2 = H$
2 $R_1 = R_2 = CH_3$
3 $R_1 = H$, $R_2 = CH_3$
4 $R_1 = CH_2CO_2H$, $R_2 = H$
5 $R_1 = CH_2CH_2CO_2H$, $R_2 = H$
6 $R_1 = CH_2CH_2CH_2CO_2H$, $R_2 = H$
7 $R_1 = CH_2CH_2OSO_3Na$, $R_2 = H$
8 $R_1 = CH_2CH_2OPO_3Na_2$, $R_2 = H$



9 R = NH_2
10 R = NHAc



11 $R_1 = R_2 = H$
12 $R_1 = CH_2CO_2H$, $R_2 = H$
13 $R_1 = H$, $R_2 = CH_2CO_2H$

Figure 1.6 *Structure of synthetic di- and trisaccharide analogs of* P^k*-trisaccharide* [80] *The atoms and positions in β-Gal residue of trisaccharide are labelled with prime (') and those for α-Gal residue are labelled with double prime (").*

Bundle and co-workers have designed Stx inhibitors based on an examination of the crystal structure of the B pentamer complexed with the Gb_3 trisaccharide analog.[79, 80] The synthesis and biological activity of P^k-trisaccharide derivatives and disaccharide analogs of the terminal galabiose have been reported (Figure 1.6).

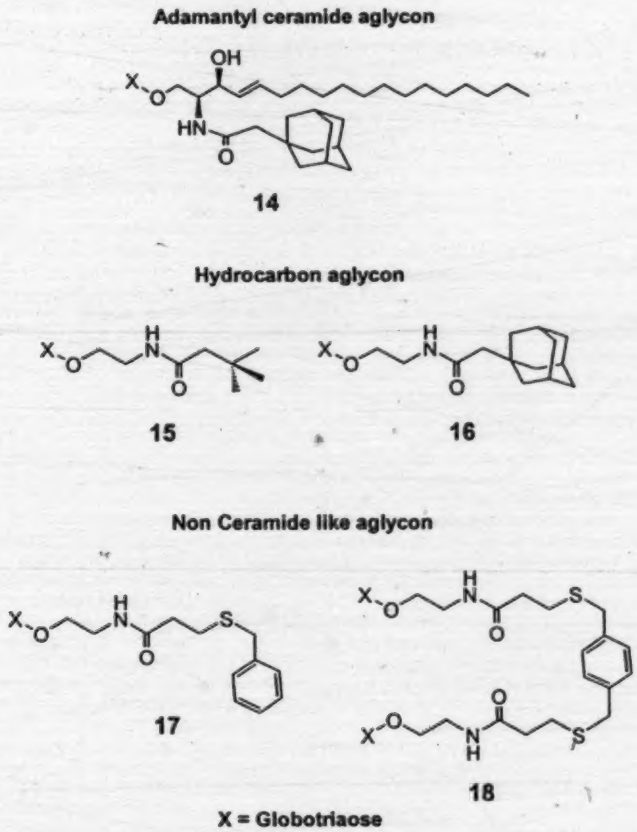


Figure 1.7 *Structure of synthetic aglycon analogs* [81]

When the modification at the O-2′ position of galabiose was made, it has been shown that the inhibitory power of carboxyalkyl galabiose analogs was higher than

that of the unmodified galabioside. However, the modification of galabiose at the O-2′ position at the trisaccharide level did not result in an increase in the inhibitory activity.

Binding of verotoxin to a series of galabiose or globotriose containing synthetic glycolipids has been studied by Lingwood and co-workers.[78] The result showed that the binding of the toxin to receptors changes dramatically with the nature of the lipid moiety. Thus, the lipid moiety was shown to be one of the determinants of inhibitory power. The research was designed employing water-soluble univalent glycosphingolipid mimics that retain the functional properties of the natural ligand,

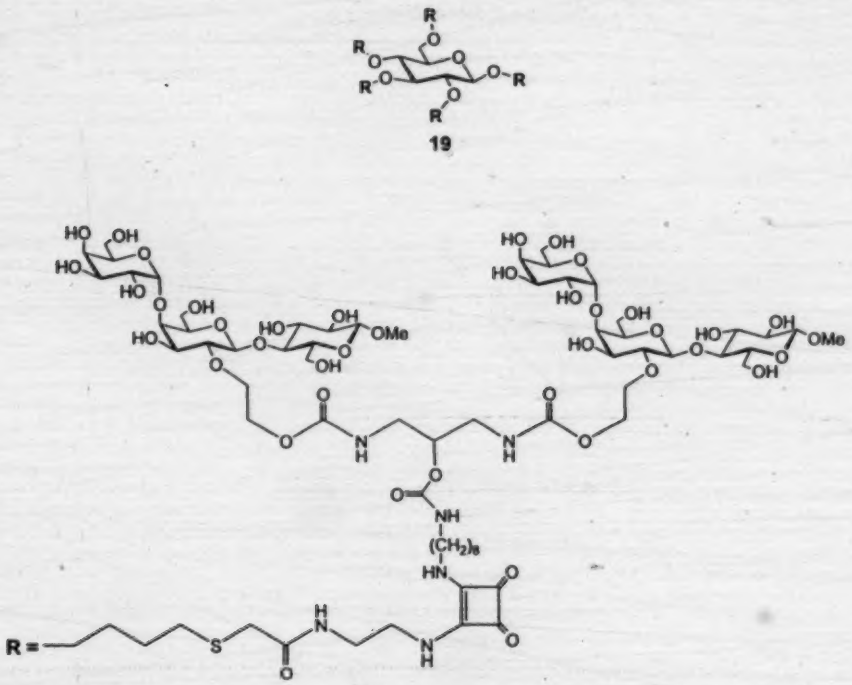


Figure 1.8 *Structure of decameric STARFISH*

while showing better binding strength, and being simpler to synthesize. Adamantyl-Gb_3 showed significantly enhanced water solubility and a thousand times increased inhibition compare to natural Gb_3 (Figure 1.7).[78, 81, 82]

Monovalent protein-carbohydrate interactions are usually weak ($K_a=10^{3-4}$ M^{-1}). This low affinity can be compensated for by a multivalent array of carbohydrate epitopes.[83, 84] The improved binding and specificity are the result of multivalent interactions. Particularly, multivalent interactions can be collectively much stronger than simple addition of corresponding univalent interactions. Ligand multivalency increases avidity. The number of carbohydrate residues, spacing and orientation are important for the adequate interaction.[85] By optimizing spacer, orientation and multivalency Bundle and co-workers achieved impressive results.[28]

The doughnut shape of the B pentamer presents a pseudo-symmetrical display of five identical carbohydrate binding sites on a single face (Figure 1.5). The desired multivalent binding interactions could be achieved by tethering five identical ligands so that simultaneous multivalent interaction was possible. The result of inhibitory activity was one to ten-million-fold higher than that of the univalent P^k ligand.

1.4 Objective of current research

One important function of carbohydrates in biological systems is to provide binding sites for different types of viruses, bacteria, and toxins. If prevention of protein-carbohydrate binding can be achieved, diseases caused by pathogens (viruses, bacteria, toxins, and other organisms) might be avoided. Thus, understanding the selectivity and driving forces for the binding of a carbohydrate to a protein is of the

utmost importance since significant evidence has accumulated that carbohydrate-protein complexes play a critical role in the maintenance of biological processes.[64]

The research described in the following chapters was aimed at elucidating the interactions between carbohydrate (P^k antigen) and protein (Shiga toxin family). Our efforts were focused on the synthesis of the P^k-trisaccharide and deoxy analogs of the galabiose moiety (α-Gal*p*-(1→4)-β-Gal*p*; the common epitope of the natural P^k antigen) for use in probing carbohydrate-protein binding sites. The analogs were selected based on our current knowledge of the binding sites on the Shiga toxins. The intention was to use deoxy analogs to obtain further information on the binding site of Shiga toxin. As part of this approach we intended to use Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) to observe the gas phase binding of native P^k-trisaccharide and seven mono- deoxy trisaccharide analogs.

Chapter 2

Synthesis of P^k-trisaccharide and Deoxy Analogs

2.1 Synthetic compounds for evaluation of binding activity

The P^k-trisaccharide, which is the receptor for Shiga toxins (Stxs), and its 7 mono-deoxy trisaccharide analogs (Figure 2.1) were synthesized to evaluate their binding activity with the B subunit of Stxs. With the exception of compound **20**, in which the α–(1→4) linkage was constructed enzymatically,[86] all of the α–(1→4) glycosidic

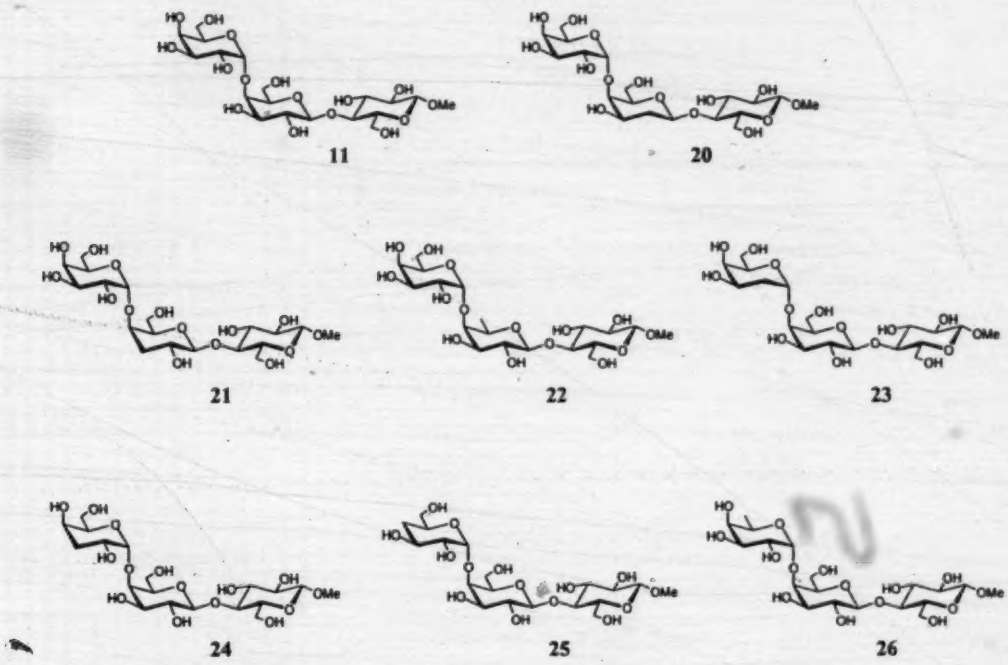


Figure 2.1 *Structure of the P^k-trisaccharide and deoxy analogs*

linkages were synthesized using chemical methods. Compounds **21**, **24** and **25** were synthesized using the Barton-McCombie deoxygenation method. Reductive deoxygenation of sulfonate ester with lithium aluminium hydride produced compounds **22** and **26**.

2.2 General synthesis of oligosaccharides

2.2.1 Background

The glycosidic linkage is chemically synthesized via the displacement of a leaving group at the anomeric center by the hydroxyl group of a partially protected sugar or alcohol.

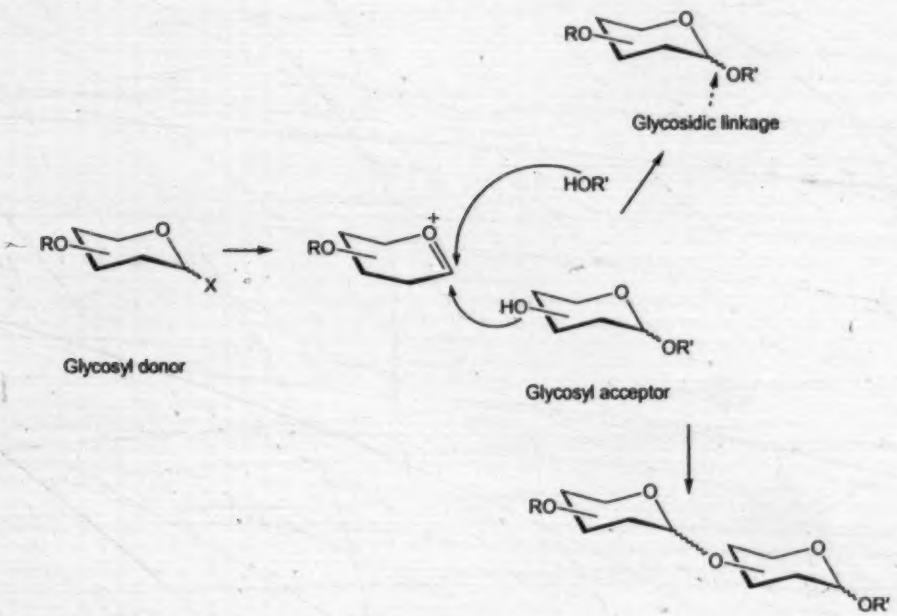


Figure 2.2 *Formation of glycosidic linkage between a glycosyl donor and acceptor*

The sugar unit containing the leaving group is called the glycosyl donor, whereas the residue bearing the attacking nucleophile, a hydroxyl group, is called the glycosyl acceptor (Figure 2.2). In 1901, the Koenigs-Knorr reaction employing glycosyl halides as donors was developed.[87] This classical method requires different heavy metals as activating agents.[88, 89] Since then many different types of glycosyl donors have been developed for the use in glycosylation reactions (Figure 2.3). Another widely used glycosyl donor is a trichloroacetimidate, which is prepared by the reaction of the anomeric alkoxy oxygen atom of a sugar with trichloroacetonitrile in the presence of a base.[90] This type of donor generally provides good yield in the glycosylation reaction under mildly acidic conditions. Thioglycosides are versatile donors due to their stability to hydrolysis. Activation by thiophilic reagents creates a reactive glycosyl donor. Furthermore, thioglycosides can be readily converted to other glycosyl donors.[91] Glycals are also very efficient donors for the synthesis of 2-deoxy glycosides.[92, 93]

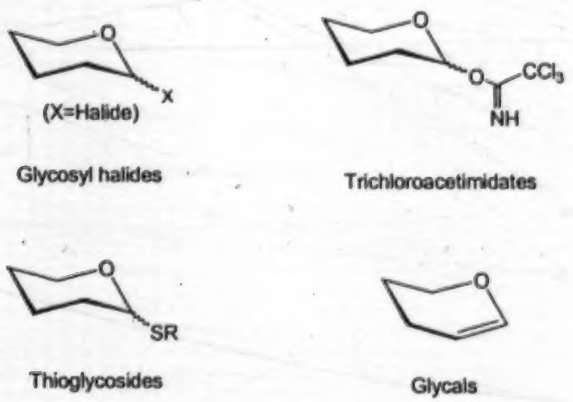


Figure 2.3 *Glycosyl donors in formation of glycosyl linkages*

Glycosyl donors can be divided into two categories: armed donors and disarmed donors, which differ in electron withdrawing properties of the protecting group at 2-OH position.[94] The lower reactivity of disarmed donors is a consequence of destabilization of the transition state that is structurally similar to the positively charged oxocarbenium ion intermediate of the glycosylation reaction by a neighboring electron withdrawing group such as esters (Figure 2.4). In contrast, the intermediate or transition state of an armed donor can be stabilized by the electron donating nature of ether-type protecting groups.

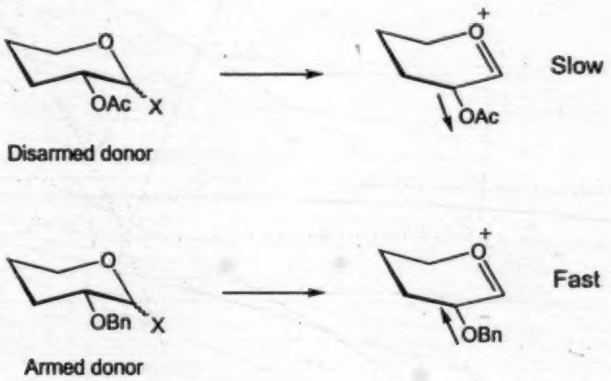


Figure 2.4 *Armed and disarmed donor*

The reactivity of acceptors depends on the nucleophilicity of the hydroxyl group in the acceptors. Primary hydroxyl groups are more reactive than secondary hydroxyl groups. Electron withdrawing groups in close proximity diminish the reactivity by decreasing the nucleophilicity of the hydroxyl group on the acceptor.

Moreover, steric hindrance and the orientation of the hydroxyl group in the acceptor also influence its reactivity.

The anomeric linkages are classified as α- and β-linkages by the relative configuration at C-1 and the ring stereogenic center furthest from C-1 and as *cis*- and *trans*- linkages by the relative configuration at C-1 and C-2 in the resulting glycoside (Figure 2.5). Usually, formation of 1,2-*trans* linkages is easier than formation of 1,2-*cis* linkages.[95] Synthetic difficulty increases in the following order: 1,2-*trans*-α <1,2-*trans*-β <1,2-*cis*-α <1,2-*cis*-β. While chemical synthesis of 1,2-*trans*-α and 1,2-*trans*-β ligands is relatively straightforward the synthesis of 1,2-*cis*-α and 1,2-*cis*-β still remain a synthetic challenge with 1,2-*cis*-β being the most difficult.

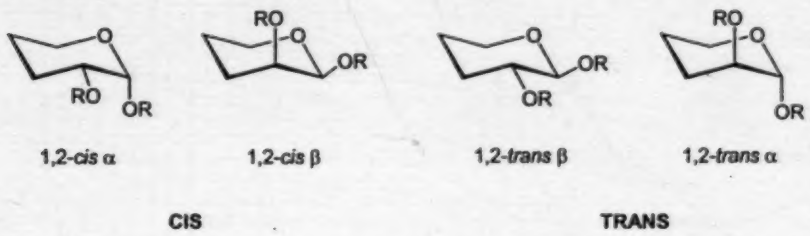


Figure 2.5 *Types of glycosidic linkages*

2.2.2 Preparation of 1,2-*trans* linkages by neighboring group participation

1,2-*Trans* linkages are readily synthesized utilizing the participation of a neighbouring group at the C-2 position. After the activation of the anomeric leaving group X (Figure 2.6), the reaction proceeds via an oxocarbenium ion intermediate. If there is a participating substituent at C-2 such as an acyl group, a cyclic carbonium

(dioxolenium) intermediate will form, which will shield its cis-face to an incoming nucleophile, resulting in the formation of a 1,2-*trans* linkage. Because the attack of the nucleophile on the dioxolenium carbon atom, which bears a formal positive charge, is kinetically more favored, orthoesters are often the intermediates in these reactions. However, the orthoester bond formation is reversible under acidic conditions and they are slowly converted to glycosides.[96]

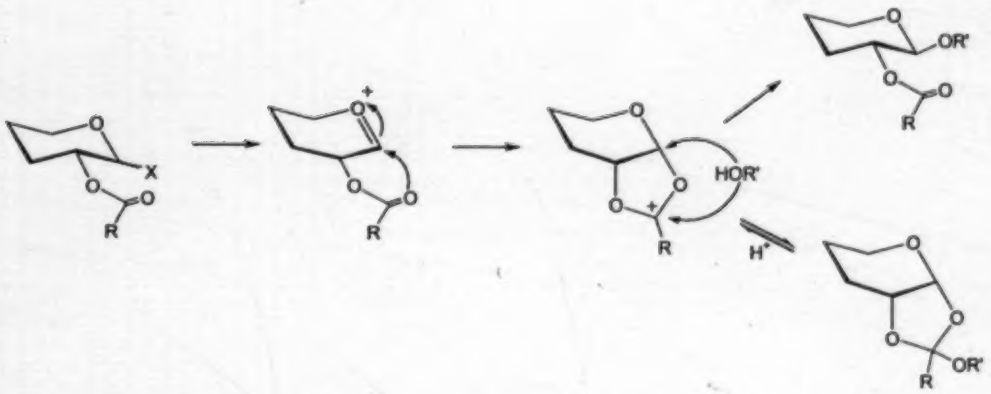


Figure 2.6 *Proposed mechanism for participating group*

The Koenigs-Knorr method employs glycosyl bromides or chlorides as donors and heavy metal salts are used as activating reagents. Initially, insoluble activators such as Ag_2O and Ag_2CO_3 were used.[87] Promoters such as $HgBr_2$ and $Hg(CN)_2$ soluble in organic solvent were later introduced by Helferich.[97] Heterogeneous processes with insoluble activators can be described as "push-pull" mechanism (Fig

2.7).[95] The utility of the Koenigs-Knorr method is limited by the toxicity of heavy metal promoters and the intrinsic low stability of most glycosyl halides.

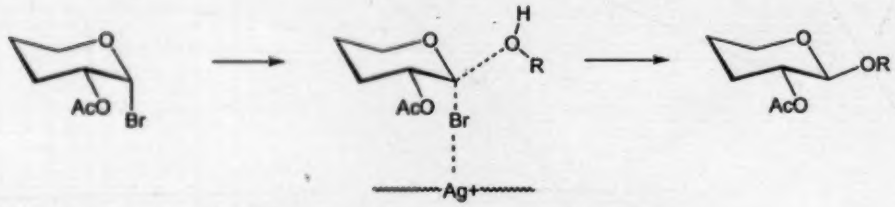


Figure 2.7 *The use of activator for 1,2-trans glycoside formation*

In the case of a non-participating group the solvent also plays an important role in the stereochemical outcome of the glycosylation reaction. Usually, solvents such as dichloromethane or ether are unavailable to form stable intermediates with glycosyl cation. In this case the stereoselectivity of the reaction is controlled by the anomeric effect and an α-glycoside is formed preferentially. Some solvents such as acetonitrile form a complex with the oxonium ion intermediate affecting the direction of the incoming alcohol (Figure 2.8).[98, 99] This is explained by the generation of an α-nitrilium intermediate. For this reason, β-glycosides can be selectively obtained in acetonitrile at low temperatures.

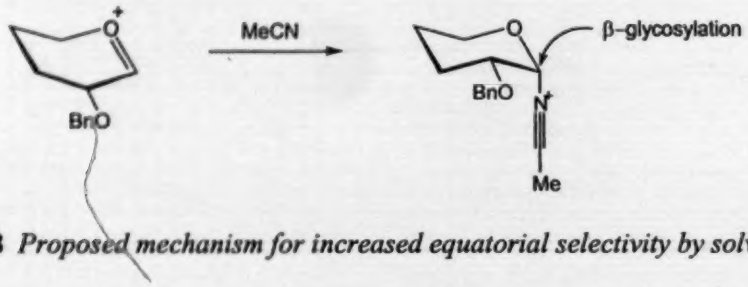


Figure 2.8 *Proposed mechanism for increased equatorial selectivity by solvent*

2.2.3 Preparation of 1,2-*cis*-α linkages

Generally, a non-participating alkyl group at C-2 position promotes the formation of both 1,2-*trans*-β and 1,2-*cis*-α glycosides. Lemieux[100] introduced the halide ion catalysis method, where the halide is either I or Br, which controls the anomeric selectivity for armed donors bearing a non-participating group at C-2. This procedure gives mainly 1,2-*cis*-α-glycosides as glycosylation products. The proposed mechanism of the reaction involves equilibration between α- and β-forms of the glycosyl halide prior to nucleophilic attack by the alcohol.

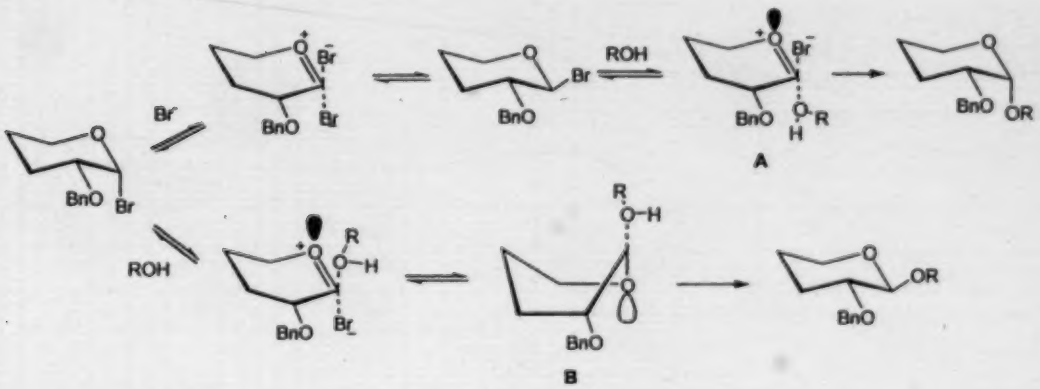


Figure 2.9 *Proposed mechanism for preparation of α-glycosides.*

The equilibrium is catalysed by the halide ion from a tetraalkylammonium halide.[95] The β-halide, which is thermodynamically less stable because of the anomeric effect, reacts more rapidly than the α-halide. The C-Br bond of the β-halide

must be in antiperiplanar arrangement for the incoming alcohol and the ring oxygen lone pair electrons. To establish an antiperiplanar arrangement, a conformational change is necessary. The transition state of **A** is lower in energy than that of **B**. As the difference in reaction rate between **A** and **B** is large enough, α-glycosides are formed as major products.

The use of glycosyl fluorides as glycosyl donors with $SnCl_2$-$AgClO_4$ as a promoter is a good alternative method for the synthesis of 1,2-*cis* glycosides.[101] The advantages of the glycosyl fluoride as a glycosyl donor are higher thermal and chemical stability over other glycosyl halides. Activators of glycosyl fluorides such as $LiClO_4$,[102] $La(ClO_4)_3$,[103] $Yb(OTf)_3$,[104] SO_4/ZrO_2,[105] TfOH,[106, 107] or $HClO_4$[108] are applied for stereoselective 1,2-*cis* glycosylation.

2.2.4 The trichloroacetimidate method

Glycosylation with anomeric trichloroacetimidate donors was introduced by Schmidt and co-workers in 1980[109] as an alternative to glycosyl halides. The reaction of 1-OH sugar derivatives with trichloroacetonitrile in the presence of an appropriate base gives the stable trichloroacetimidate glycosyl donors[88, 90, 95] transforming the anomeric oxygen into a good leaving group. NaH is a good base for the formation of a thermodynamically stable axial trichloroacetimidate. In the presence of a weak base, such as K_2CO_3, the equatorial trichloroacetimidate is formed very rapidly. Other bases such as DBU often result in anomeric mixture of isomers. The reaction of these donors usually gives good yields of glycosides under mild acidic conditions employing BF_3•OEt_2 or TMSOTf.[110] This method is used not only in the synthesis of

oligosaccharides, but also in the synthesis of glycoconjugate natural products, for example glycosphingolipids[111] and gangliosides.[112]

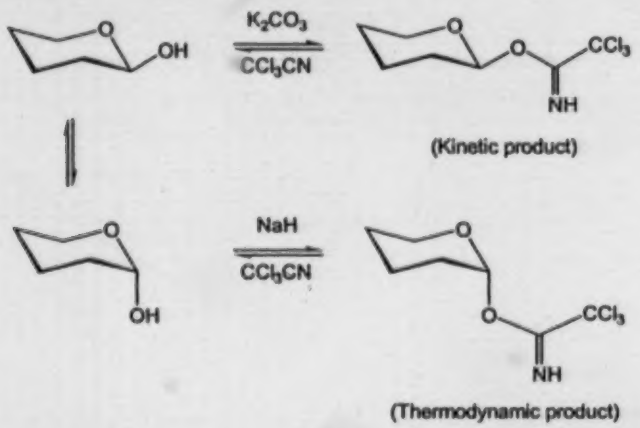


Figure 2.10 *Formation of α and β–trichloroacetimidate using different bases*

2.2.5 The thioglycoside method

Thioglycosides have become frequently used glycosyl donors. The sulfur has a mild nucleophilicity and can react with thiophilic activators[113] to provide the intermediate sulfonium ion that eliminates the corresponding thioalkyl halide to produce an oxocarbenium intermediate that is attacked by a nucleophile to produce a glycoside.

Figure 2.11 *Formation of intermediate sulfonium ion*

The thiogroup in the anomeric position can function as either a ether protecting group of the anomeric position or a good leaving group, which makes thioglycosides attractive building blocks for the synthesis of oligosaccharides.[114] Moreover, thioglycosides are very versatile and can be converted to several other activated glycosyl donors such as halides and sulfoxides (Figure 2.12).

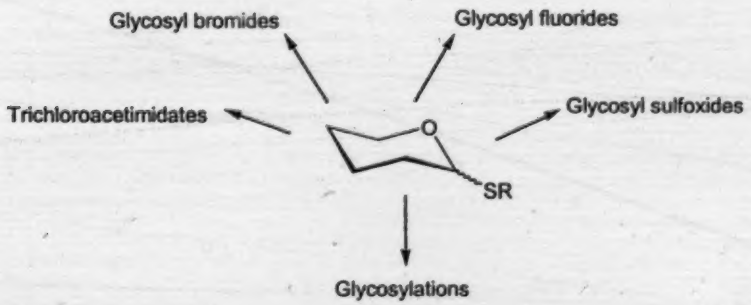


Figure 2.12 *Conversions of thioglycosides to other glycosyl donors*

2.2.6 Enzymatic glycoside synthesis

The chemical synthesis of oligosaccharides usually requires extensive protection and deprotection steps. The application of enzymes in carbohydrate synthesis can eliminate these arduous steps and, in addition, take advantage of the high regio- and stereoselectivity of enzyme reactions. Thus, enzymatic synthesis of oligosaccharides has become an attractive method in carbohydrate chemistry.[115, 116] The three types of enzymes involved are glycosyltransferases, glycosidases and glycosynthases.[95, 115]

Glycosyltransferases are highly efficient catalysts for the regio- and stereoselective formation of glycosidic linkages. Glycosyltransferases are responsible

for transferring a sugar moiety of an activated donor to the glycosyl acceptor (Figure 2.13 a). Activated donors include sugar nucleotides such as UDP-glucose and UDP-galactose, and sugar phosphates. Many applications in oligosaccharide synthesis employing glycosyltransferases have been reported.[84, 117-119] However, the use of glycosyltransferases is restricted because the number of glycosyltransferases available from natural sources is limited and nucleotide sugar donors are usually expensive and hard to synthesize. By making use of recombinant gene technology, it has been possible to obtain cloned enzymes that synthesize UDP sugar donors.

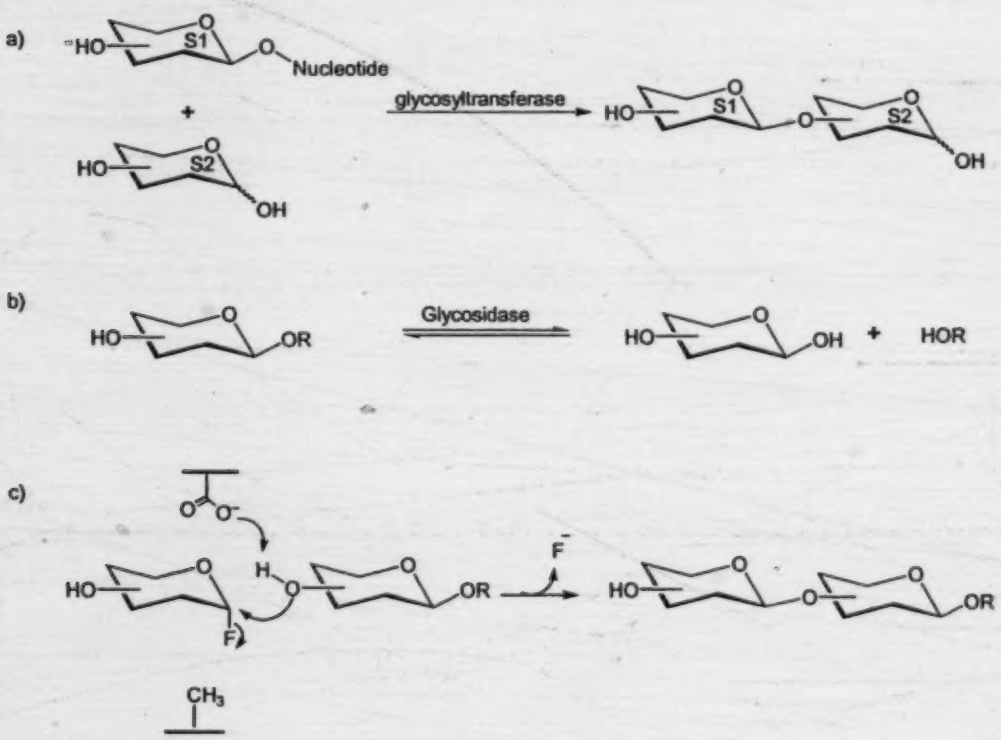


Figure 2.13 *Enzymatic glycosylation*

Glycosidases are another class of carbohydrate processing enzymes that are also used in the synthesis of glycosides. This type of enzyme usually catalyzes the cleavage of glycosidic bonds but under certain circumstances it can catalyze the reverse reaction, glycosidic bond formation, which is utilized for the synthesis of glycosides.[120] While glycosidases have the advantages of wide availability, stability, tolerance of organic solvents, and low cost compared to glycosyltransferases, they do not generally give high yields because the products are also their substrates that can undergo natural cleavage.

Glycosynthases are mutant glycosidases which can make the glycosidic linkages in good yield. In these enzymes an amino acid with a nucleophilic carboxylic side chain is replaced by a non-nucleophilic amino acid such as glycine or alanine. This allows the transition state of glycoside hydrolysis to be captured by an alcohol rather than the nucleophilic carboxyl group of the active site amino acid. Glycosynthases have been used in this way to catalyze the transfer of the sugar unit from a glycosyl fluoride to sugar acceptors (Figure 2.13 c).[121, 122]

2.3 Synthesis of P^k-trisaccharide

2.3.1a Retrosynthetic strategy for the synthesis of the P^k-trisaccharide

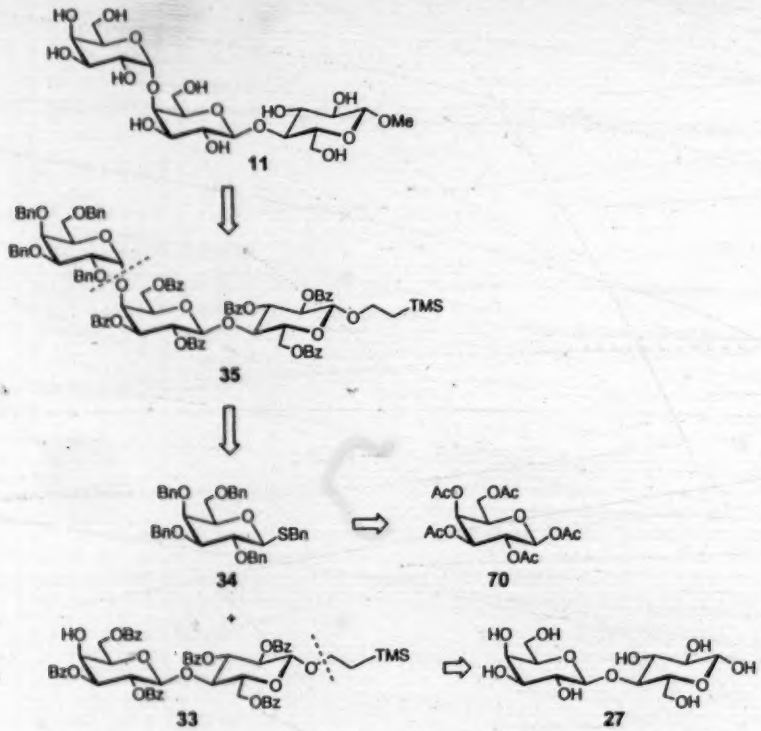
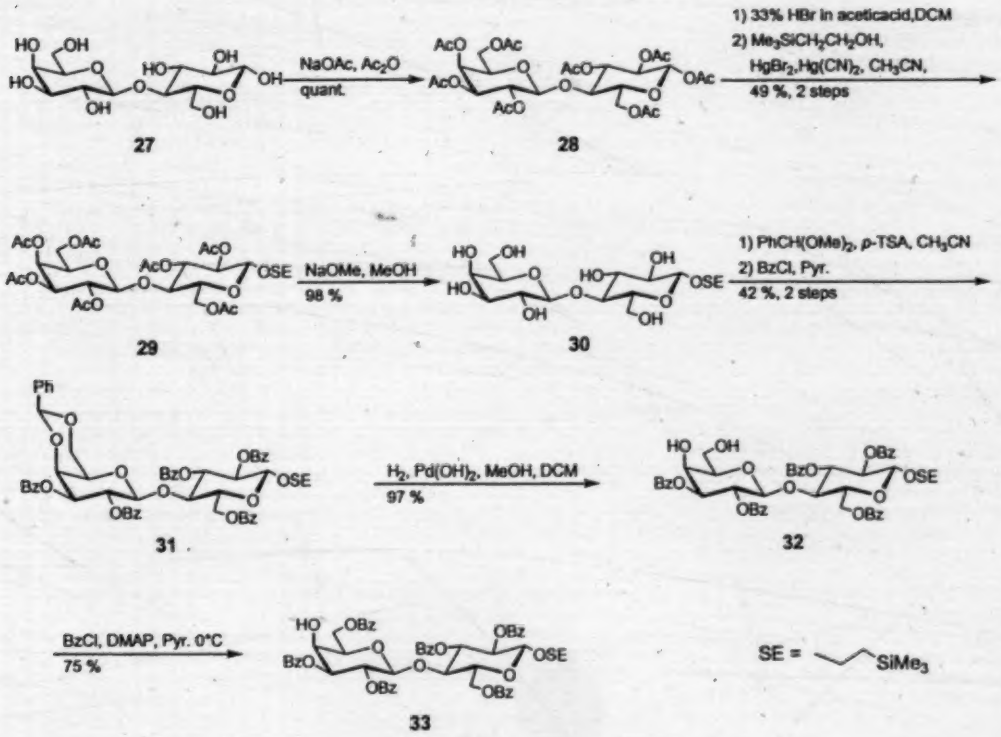


Figure 2.14 *Retrosynthesis of P^k- trisaccharide*

The target P^k-trisaccharide **11** can be prepared from the fully protected glycoside **35** that can be synthesized from the galactosyl donor **34** and lactosyl acceptor **33**. The 2-(trimethylsilyl)ethyl group was chosen as an anomeric protecting group[123, 124] in the acceptor **33** because it can be later transformed into a trichloroacetimidate donor to afford the desired methyl glycoside. The benzyl group was chosen for the protection of the remaining hydroxyl groups. The partially deprotected lactosyl acceptor **33** could be glycosylated at its free 4-OH position by a

perbenzylated thiodonor **34**. Both donor and acceptor precursors, **70** and **27**, can be made starting from commercially available D-galactose and D-lactose respectively.

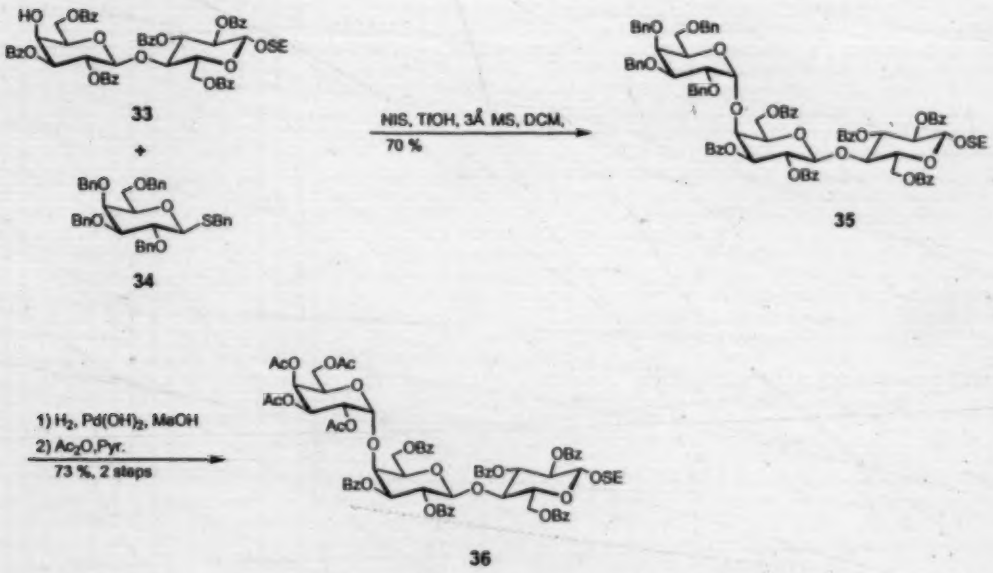
2.3.1b Synthesis of the P^k-trisaccharide



Scheme 2.1 *Synthesis of protected lactosyl acceptor 33*

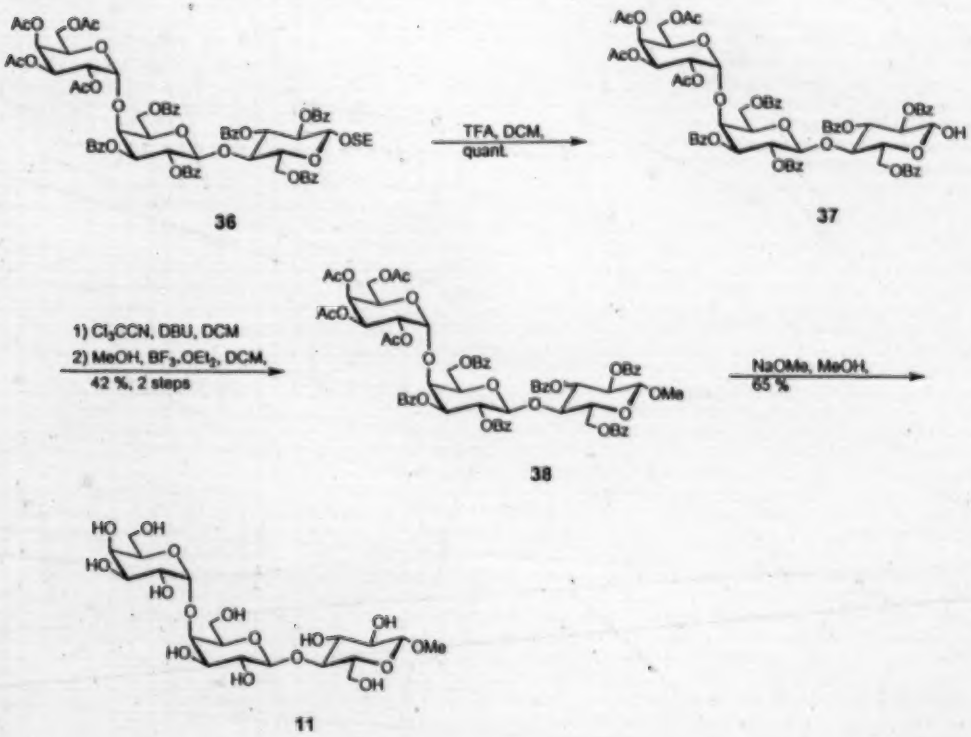
The important intermediate acceptor **33** was synthesized from lactose **27** (Scheme 2.1). The peracetylated lactose **28** was synthesized on a large scale from lactose **27** in good yield according to a known procedure.[125] Treatment of **28** with HBr in acetic acid generated the corresponding glycosyl bromide donor, which was

glycosidated with 2-(trimethylsilyl) ethanol under classical Helferich conditions to give the 2-(trimethylsilyl) ethyl (SE) glycoside **29** in 49 % yield after 2 steps.[124, 126-128] Compound **29** was subsequently deacetylated under Zemplén conditions to give **30** in 98 % yield.[124] The heptaol **30** was reacted with benzaldehyde dimethyl acetal in the presence of a catalytic amount of *p*-toluenesulfonic acid and subsequently treated with benzoyl chloride to give the fully protected derivative **31** in 42 % yield after 2 steps. The benzylidene acetal was removed using hydrogenolysis with Pearlman's catalyst to give derivative **32** in 97 % yield. The 6'-OH group of **32** was selectively benzoylated using benzoyl chloride in pyridine at 0 °C to afford the key intermediate **33** in 75 % yield.



Scheme 2.2 *Synthesis of protected trisaccharide* **36**

The glycosylation of **33** with donor **34**[129] using *N*-iodosuccinimide (NIS) and trifluoromethansulfonic acid (TfOH) furnished fully protected trisaccharide **35** in 70 % yield (Scheme 2.2). The benzyl groups of compound **35** were removed by hydrogenation to form the tetraol and subsequently acetylated with acetic anhydride in pyridine to afford protected trisaccharide **36** in 73 % yield after 2 steps.



36 **37** **38** **11**

Scheme 2.3 *Synthesis of native P^k- trisaccharide* **11**

The anomeric TMS ethyl group of **36** was successfully removed with trifluoroacetic acid in dichloromethane to give hemiacetal **37** in 92 % yield (Scheme 2.3). Conversion of this hemiacetal to trichloroacetimidate using trichloroacetonitrile

and DBU in dichloromethane followed by the glycosydation with methanol using boron trifluoride diethyl etherate as an activator afforded trisaccharide **38** in 42 % yield after 2 steps. Finally, total deprotection of compound **38** under Zemplén conditions gave the native P^k-trisaccharide **11** in 65 % yield.

2.3.2a Retrosynthetic strategy for the synthesis of 2′-, 3′- and 6′-deoxy analogs

The target compounds **20**, **21**, and **22** (Figure 2.15) can be prepared from donor **34** and corresponding monodeoxy acceptors **47**, **58**, and **64**. These acceptors can be synthesized from methyl lactoside **49** that can be prepared from D-lactose **27**.

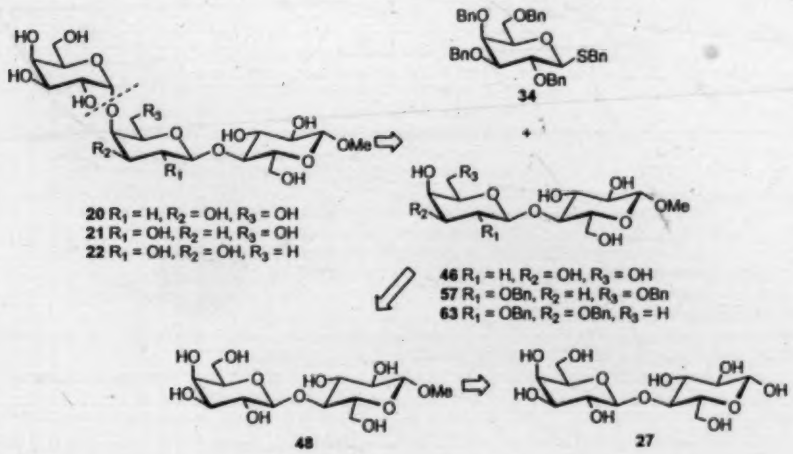
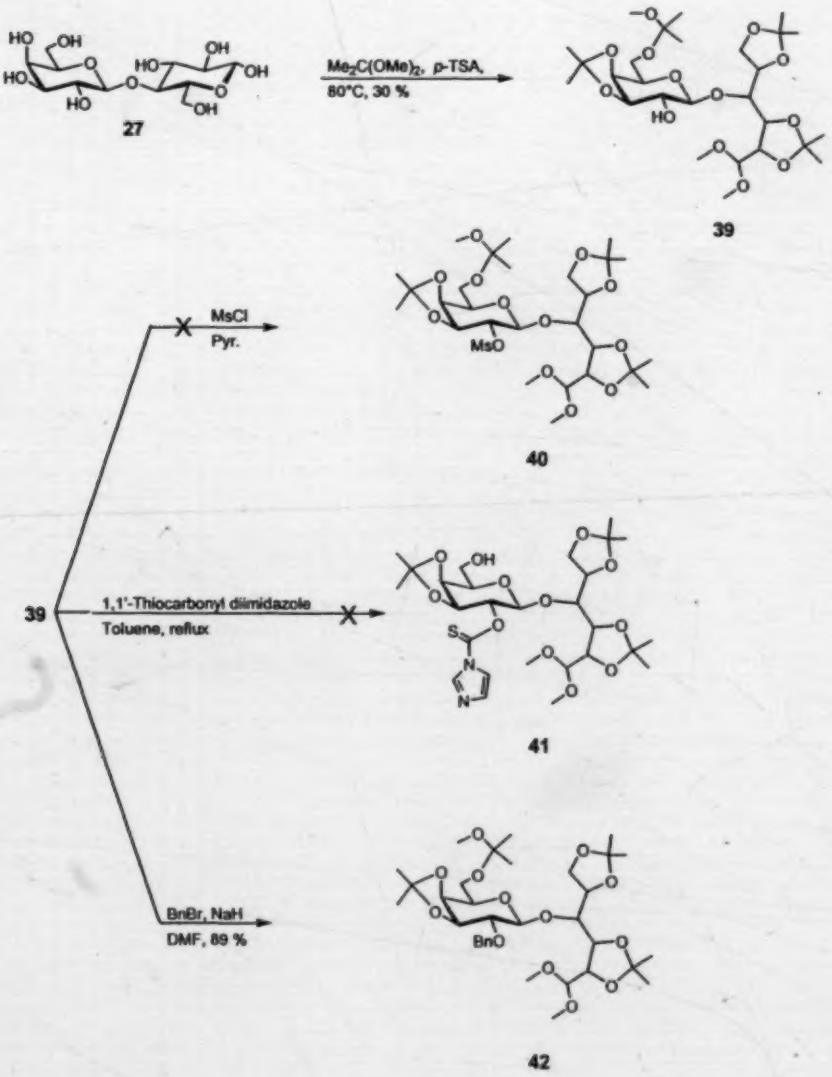


Figure 2.15 *Retrosynthesis of 2′-, 3′- and 6′-deoxy analogs*

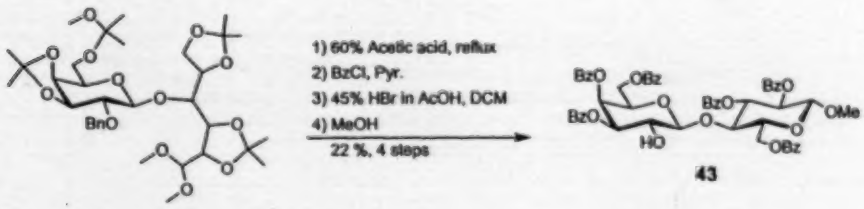
2.3.2b Synthesis of 2′-deoxy analog

In our approach towards 2′-deoxy analog **20** we envisioned the preparation of a lactose derivative that is selectively functionalized at the C-2′ position in order to undergo deoxygenation.



Scheme 2.4 *Preparation of compound* **42**

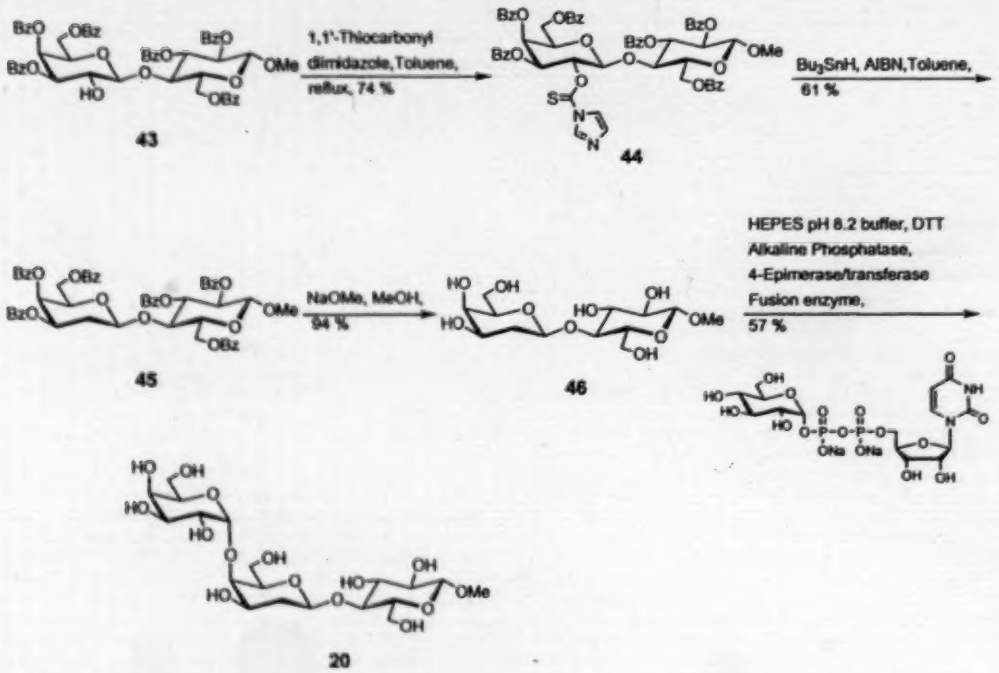
The synthesis of 2′-deoxy analog **20** started with the reaction of lactose with 2,2-dimethoxypropane. The acetonide **39**, in which the reducing glucose is fully protected in the acyclic form, was obtained in 30 % yield (Scheme 2.4).[80, 127, 128] To functionalize the C-2′ position, we examined three different protection methods. Attempts to form mesylate **40** and thiocarbamate **41** failed. Benzylation of acetonide **39** was accomplished using benzyl bromide to give compound **42** in good yield.



42 **43**

Scheme 2.5 *Synthesis of methyl glycoside 43*

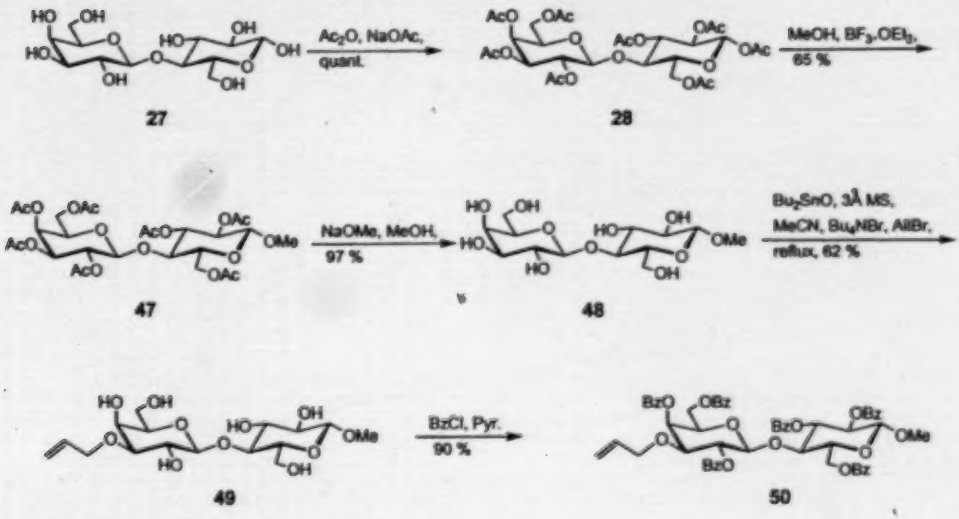
The treatment of **42** with acetic acid under reflux furnished the monobenzyl protected lactose.[130] The benzoylation of the monobenzyl protected lactose with benzoyl chloride in pyridine[80] (Scheme 2.5) followed by treatment with HBr in AcOH resulted in the formation of the lactosyl bromide with removal of the benzyl group at C-2′ position. The subsequent glycosidation with methanol gave methyl glycoside **43** in 22 % yield after 4 steps.



Scheme 2.6 *Synthesis of 2'-deoxy analog* ***20***

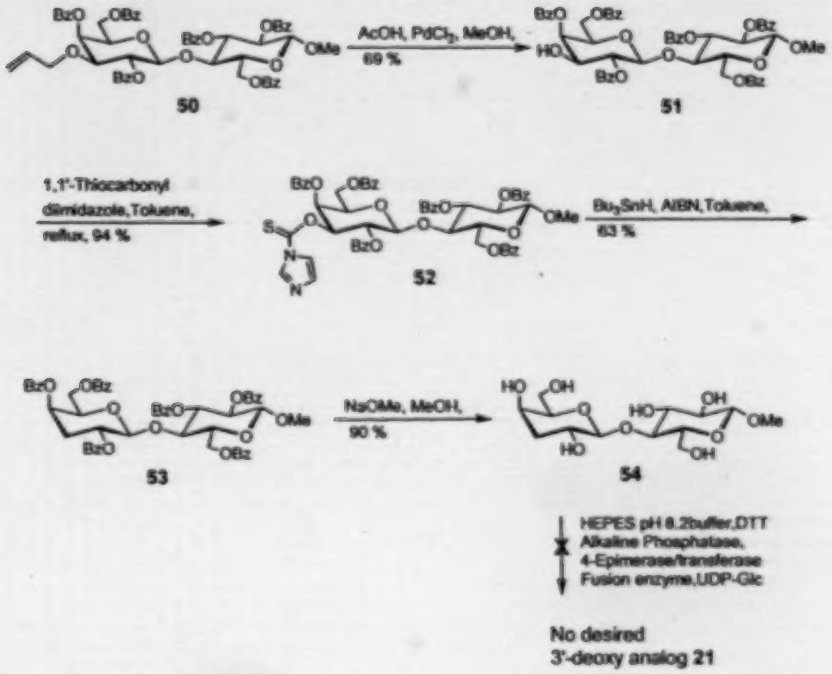
The lactoside **43** was first converted into the thiocarbamate derivative **44** in 74 % yield using 1,1'-thiocarbonyl diimidazole in toluene under reflux condition. The treatment of **44** with tributyltin hydride and AIBN in toluene afforded the 2'-deoxy derivative **45** in 61 % yield (Scheme 2.6). Subsequent debenzoylation with sodium methoxide in methanol gave 2'-deoxy lactose derivative **46** in 94 % yield. The galactosylation of **46** was performed enzymatically using UDP-Glucose and the fusion enzyme α-(1→4)-galactosyltransferase/UDP-4'-Gal-epimerase to give target compound **20** in 57 % yield.

2.3.2c Synthesis of 3′-deoxy analog



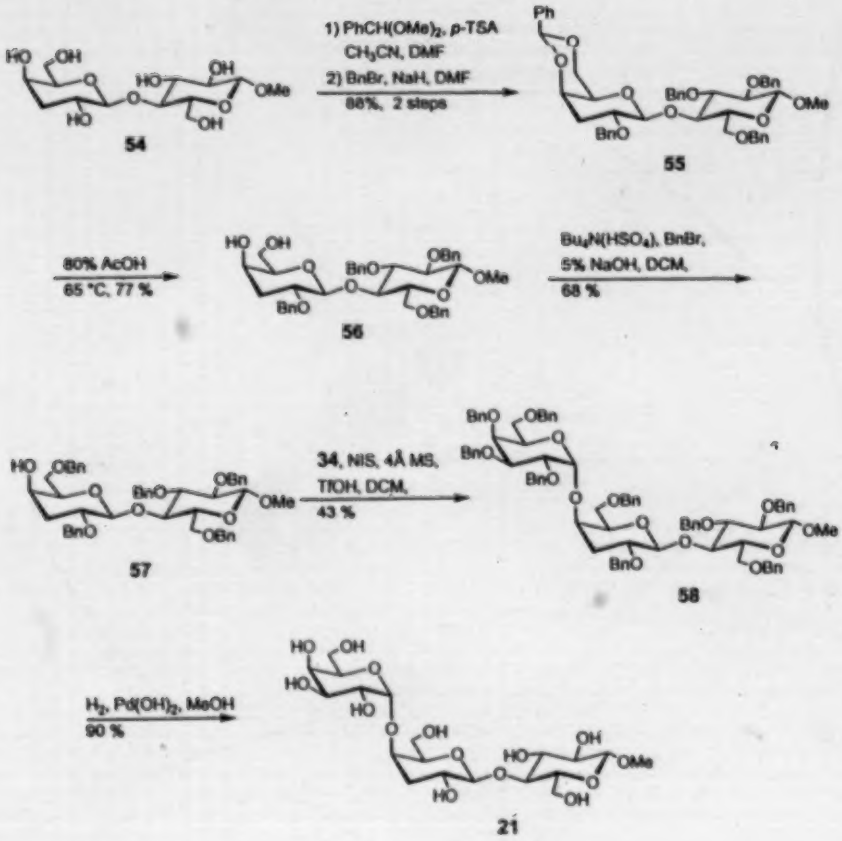
Scheme 2.7 *Synthesis of protected derivative 50*

The synthesis of 3′-deoxy P^k-trisaccharide analog **21** began with peracetylation of lactose to give octaacetate **28**[125] in quantitative yield. Compound **28** was converted into the methyl glycoside **47** using $BF_3 \cdot OEt_2$ as the Lewis acid in 65 % yield (Scheme 2.7). The methyl glycoside **47** was deacetylated to give heptol **48**[131] in 97 % yield. The C-3′ position of **48** was selectively allylated using dibutyltin oxide and allyl bromide to afford hexaol **49** in 62 % yield over 2 steps. The remaining hydroxyl groups of **49** were benzoylated using benzoyl chloride in pyridine to give fully protected derivative **50** in 90 % yield.



Scheme 2.8 *Synthesis of 3'-deoxy glycosyl donor 54*

Deallylation of compound **50** with palladium (II) chloride and acetic acid in methanol gave alcohol **51** in 69 % yield (Scheme 2.8). Compound **51** was reacted with 1,1'-thiocarbonyl diimidazole in toluene under reflux and the resulting carbamate derivative **52** was deoxygenated with tributyltin hydride and AIBN in toluene to give the 3'-deoxy disaccharide **53** in 67 % yield. The ester protecting groups of **53** were removed by sodium methoxide in methanol to give the unprotected glycoside **54**. An attempt was made to synthesize the desired 3'-deoxy trisaccharide **21** using enzymatic glycosylation. However, the reaction failed to produce the target compound indicating that the 3'-OH group of the lactose substrate is essential for enzymatic activity.

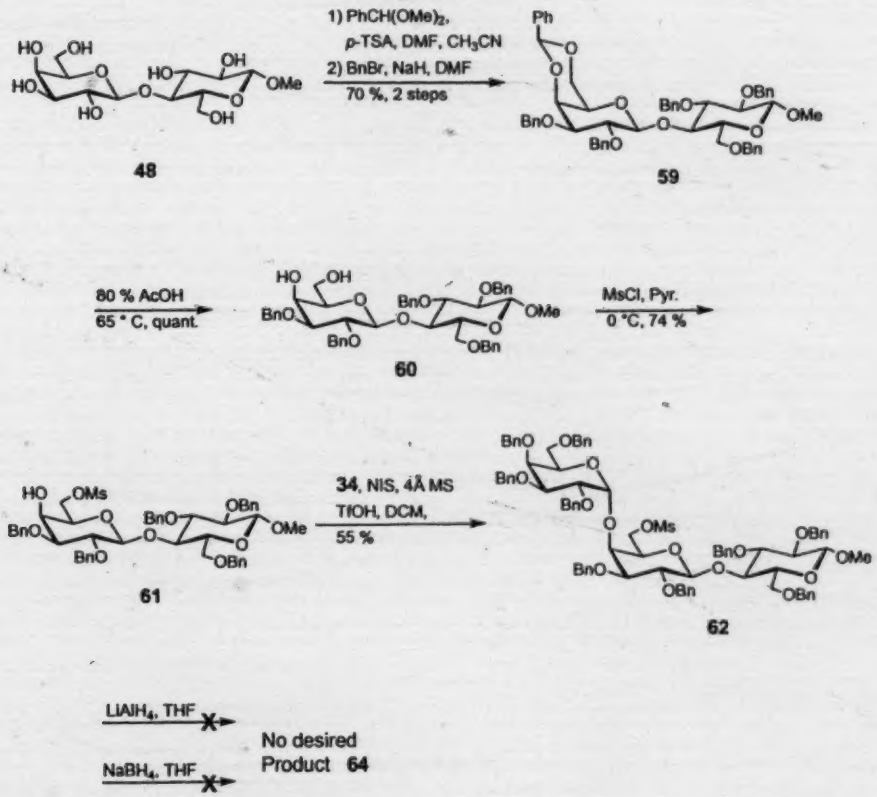


Scheme 2.9 *Synthesis of 3'-deoxy P^1-trisaccharide analog 21*

An alternative strategy was thus required for the synthesis of the 3'-deoxy trisaccharide **21** (Scheme 2.9). The 4',6'-*O*-benzylidene was installed with benzaldehyde dimethylacetal under acidic conditions, and subsequent benzylation with benzyl bromide and sodium hydride in DMF gave compound **55** in 88 % yields after 2 steps. Opening of the benzylidene ring was carried out with 80% acetic acid to afford diol **56** in 77 % yield. The treatment of **56** with tetrabutylammonium hydrogensulfate and benzyl bromide under basic conditions in the presence of a phase

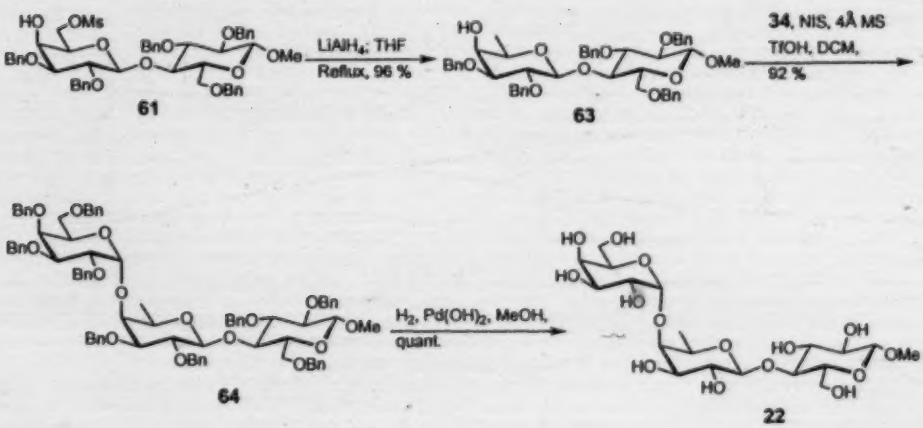
transfer catalyst afforded the 6′-protected derivative **57** in 68 % yield and good selectivity.[132] The glycosylation of **57** with **34** under the same conditions that were used to prepare trisaccharide **35** gave fully protected trisaccharied **58**. Hydrogenolysis using palladium hydroxide as a catalyst gave the final 3′-deoxy P^k-trisaccharide **21** in 90 % yield.

2.3.2d Synthesis of 6′-deoxy analog



Scheme 2.10 *Synthesis of derivative 62*

The synthesis of 6′-deoxy P^k-trisaccharide **22** started from methyl lactoside **48** (Scheme 2.10). Protection of the 4′ and 6′ positions as a benzylidene acetal followed by benzylation of the remaining alcohols yielded the fully protected derivative **59** in 70 % yield after 2 steps. Acidic hydrolysis of the benzylidene acetal, followed by selective mesylation of the resulting diol **60** with mesyl chloride at 0 °C gave compound **61** in 72 % yield after 2 steps. This acceptor was glycosylated with glycosyl donor **34** using NIS and TfOH to get fully protected trisaccharide **62** in 55 % yield. The attempted use of lithium aluminum hydride or sodium borohydride for the removal of the mesyl group of **62** failed to afford the desired product.

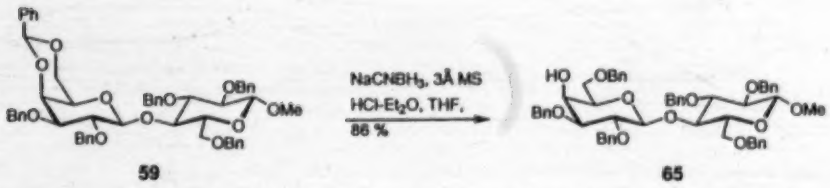


Scheme 2.11 *Synthesis of 6′-deoxy P^k-trisaccharide analog 22*

In an alternative strategy, the mesyl group was removed in the less hindered disaccharide **61** using lithium aluminum hydride to produce lactoside **63** in 96 % yield. The alcohol **63** was then glycosylated with donor **34** to give trisaccharide **64** in

92 % yield. The subsequent debenzylation of **64** led to the final 6′-deoxy P^k-trisaccharide **22** in quantitative yield.

2.3.3 Synthesis of acceptor for 2″-, 3″-, 4″- and 6″-deoxy analogs



Scheme 2.12 *Synthesis of acceptor 65 for 2"-, 3"-, 4"- and 6"-deoxy analogs*

The key acceptor for the synthesis of the 2″-, 3″-, 4″- and 6″-deoxy analogs is methyl glycoside derivative **65**. The acceptor was synthesized from lactose using the same reaction sequence as previously described for the 3′- and 6′-deoxy analogs to afford **59**. Subsequently, regioselective ring-opening of the benzylidene acetal[133] in **59** yielded the desired acceptor **65** in 86 % yield.

2.3.4a Retrosynthetic strategy of 2″-, 3″-, 4″- and 6″-deoxy analogs

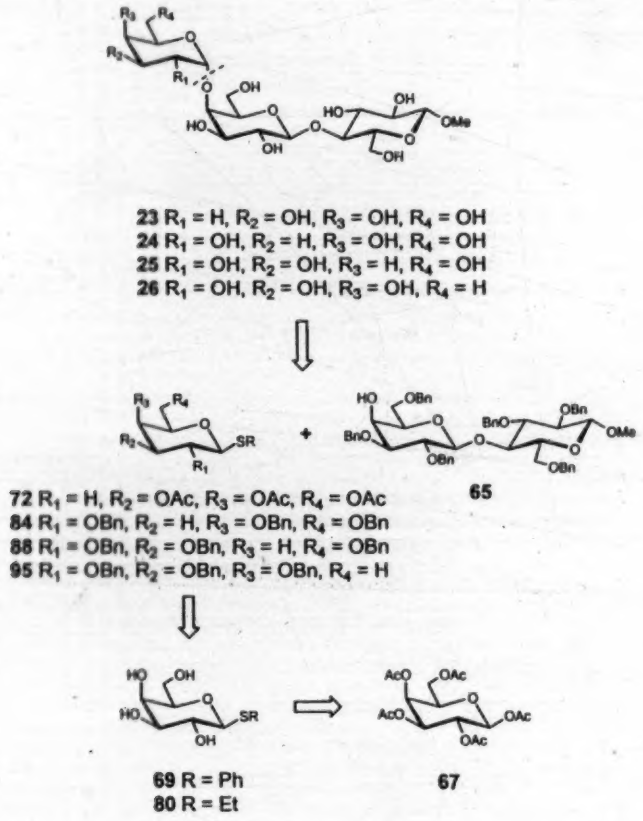
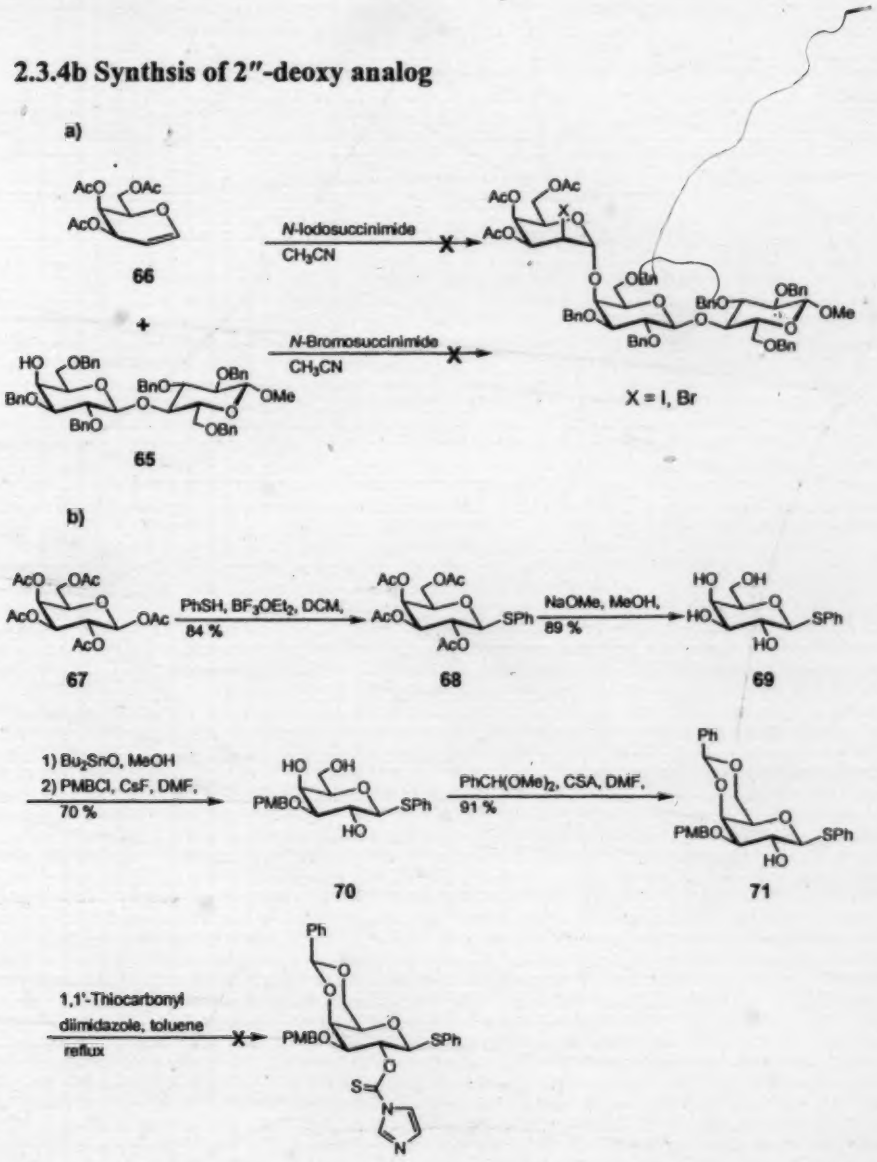

23 R1 = H, R2 = OH, R3 = OH, R4 = OH
24 R1 = OH, R2 = H, R3 = OH, R4 = OH
25 R1 = OH, R2 = OH, R3 = H, R4 = OH
26 R1 = OH, R2 = OH, R3 = OH, R4 = H
72 R1 = H, R2 = OAc, R3 = OAc, R4 = OAc
84 R1 = OBn, R2 = H, R3 = OBn, R4 = OBn
88 R1 = OBn, R2 = OBn, R3 = H, R4 = OBn
95 R1 = OBn, R2 = OBn, R3 = OBn, R4 = H
65
69 R = Ph
80 R = Et
67

Figure 2.16 *Retrosynthesis of 2"-, 3"-, 4"-and 6"-deoxy analogs*

The retrosynthetic analysis of the 2″-, 3″-, 4″- and 6″-deoxy analogs (Figure 2.16) is similar to that of the 2′-, 3′- and 6′-deoxy analogs (Figure 2.15), which were previously discussed. The deoxy analogs **23**, **24**, **25**, and **26** (Figure 2.16) can be prepared by coupling of corresponding deoxy thioglycosides **72**, **84**, **88**, and **95** with acceptor **65**. The desired donors can be synthesized using either phenyl

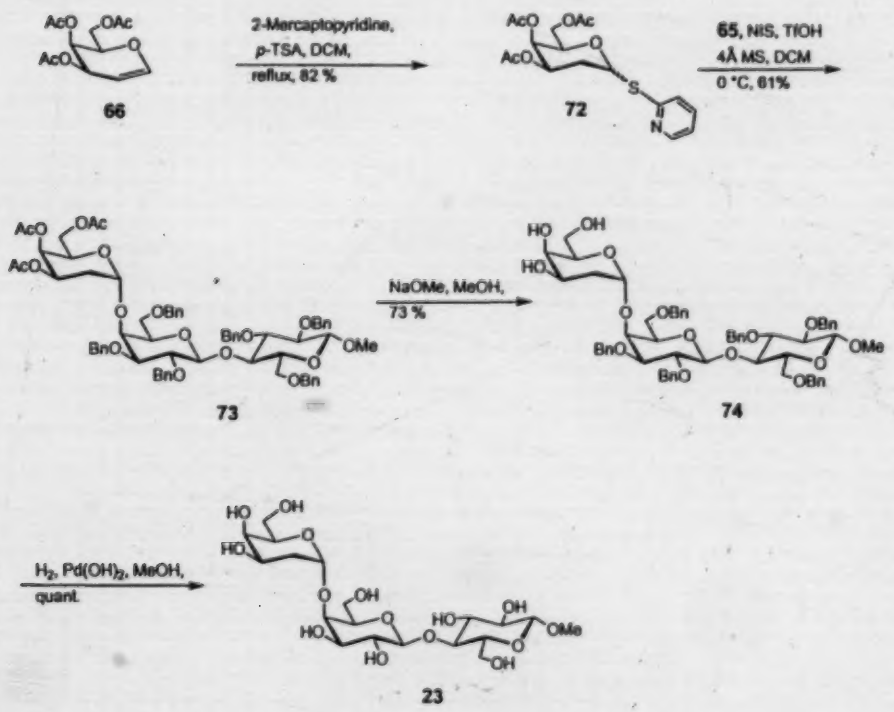
thiogalactoside **69**[134] or ethyl thiogalactoside **80**,[135] which can both be prepared from known pentaacetate **67**.

2.3.4b Synthsis of 2″-deoxy analog



Scheme 2.13 *Attempted preparation of 2″-functionalized donor*

A number of strategies were considered for the synthesis of 2"-deoxy analog **23**. Approach **a**, which employs peracetylated galactal **66** and acceptor **65** using *N*-iodosuccinimide or *N*-bromosuccinimide as a promoter, failed (Scheme 2.13). Approach **b** started from galactose pentaacetate **67**. It was initially converted to the phenyl thiogalactoside **68** under Lewis acid activation in 84 % yield. The compound **68** was then deacetylated with sodium methoxide to give tetraol **69** in 89 % yield. The C-3 position of **69** was selectively protected with the 4-methoxybenzyl group to give triol **70** in 70 % yield. Compound **70** was reacted with benzaldehyde dimethlyacetal to produce alcohol **71** in 91 % yield.



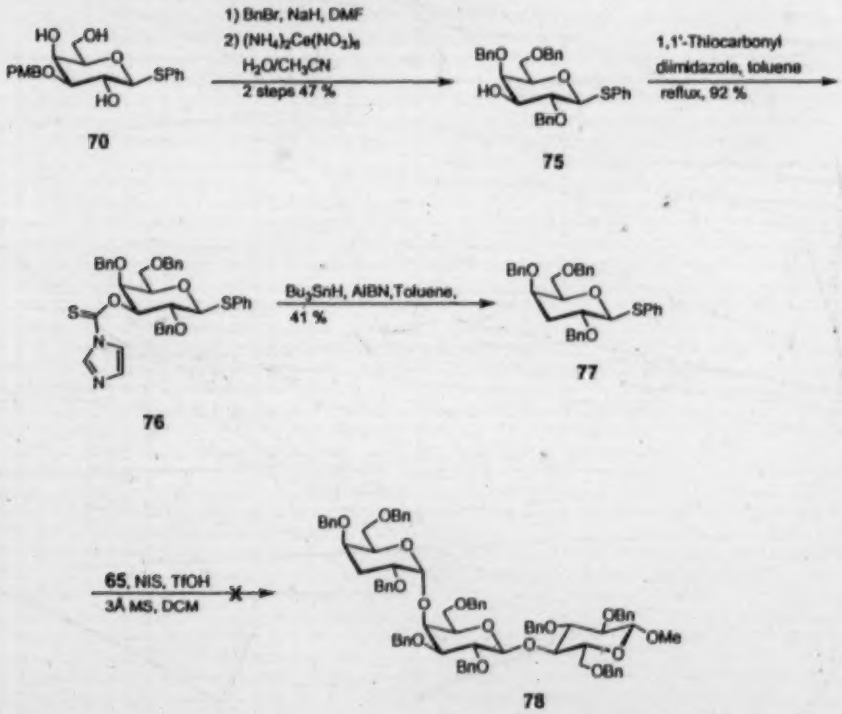
66 **72** **73** **74** **23**

Scheme 2.14 *Synthesis of 2"-deoxy P^1-trisaccharide analog 23*

However, the attempted Barton-McCombie deoxyzenation of **71** failed to afford the desired deoxy derivative.

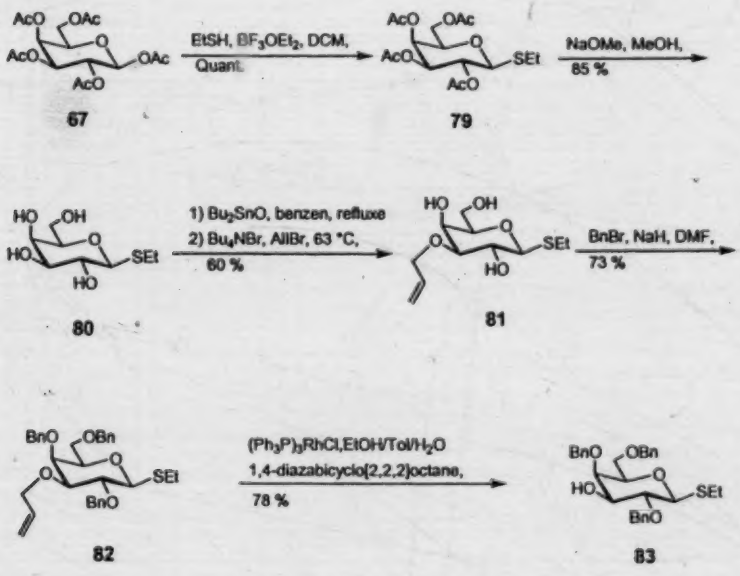
Finally, the synthesis of 2″-deoxy P^k-trisaccharide analog **23** was successfully achieved using a 2-pyridyl 2-deoxy-1-thiogalactoside derivative **72** that was obtained in 82 % yield by treatment of galactal **66** with 2-mercaptopyridine (Scheme 2.14).[136, 137] The subsequent glycosylation of acceptor **65** with **72** using NIS and TfOH as promoters afforded fully protected 2″-deoxy trisaccharide **73** in 55 % yield. The total two step deprotection of **73** yielded the target 2″-deoxy P^k-trisaccharide **23** in 73 % yield.

2.3.4c Synthesis of 3″-deoxy analog



Scheme 2.15 *Attempted synthesis of 3″-deoxy P^k-trisaccharide analog*

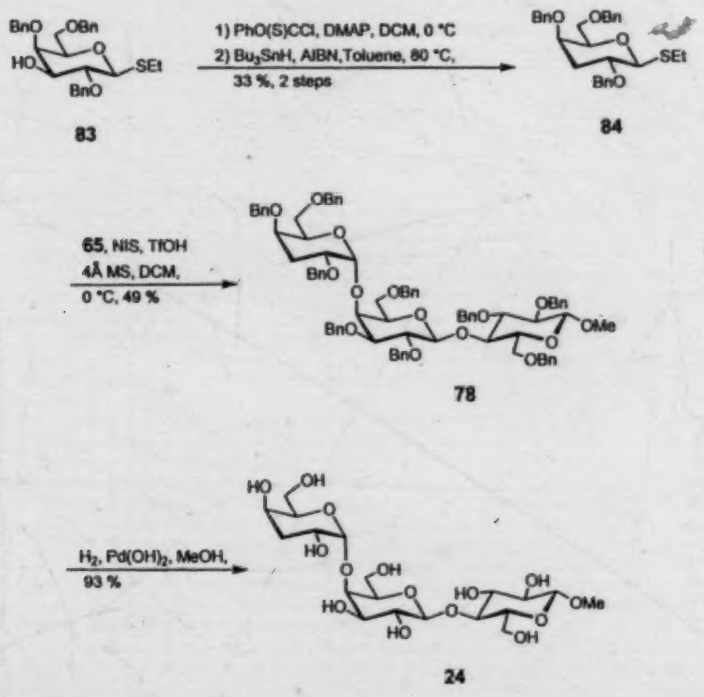
The benzylation of the hydroxyl groups of **70** followed by removal of the 4-methoxybenzyl group using CAN in water/acetonitrile gave alcohol **75** in 47 % yield after two steps (Scheme 2.15). The coupling of **75** with 1,1′-thiocarbonyl diimidazole in toluene under reflux followed by deoxygenation using tributyltin hydride and AIBN in toluene afforded the desired compound **77** in 38 % yield after 2 steps. Attempts to glycosidate donor **77** with acceptor **65** using NIS and TfOH as promoters failed (Scheme 2.15).



Scheme 2.16 *Synthesis of derivative 83*

Due to the lack of reactivity of thiophenyl donor **77**, we modified our synthetic scheme by employing a more active ethyl thioglycoside **84**. First, peracetylated galactose was converted into ethyl thioglycoside **79**[138] using ethanethiol and Lewis

acid in quantitative yield. The deacetylation of **79** using sodium methoxide gave tetraol **80**[135] in 85 % yield (Scheme 2.16). An allyl group was selectively introduced at the C-3 position of **80** using dibutyltin oxide and allyl bromide to give triol **81** in 60 % yield. The subsequent benzylation of **81** with benzyl bromide and sodium hydride in DMF gave fully protected compound **82** in 73 % yield. The deprotection of compound **82** using Wilkinson's catalyst afforded derivative **83** in 78 % yield.

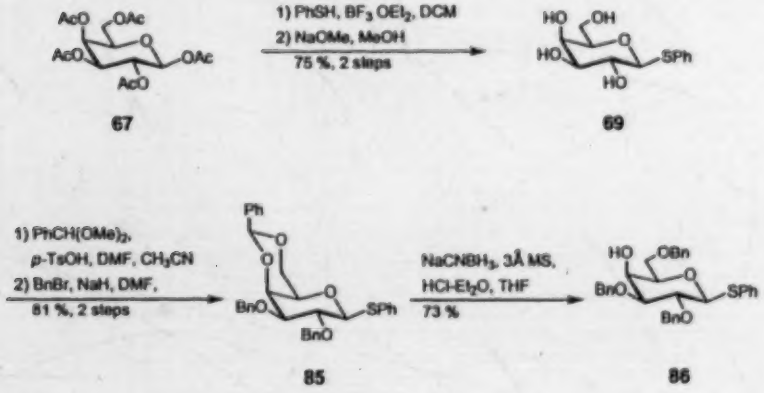


Scheme 2.17 *Synthesis of 3"-deoxy P^k-trisaccharide analog 24*

The treatment of compound **83** with phenyl thionochloroformate followed by cleavage of the resulting thiocarbamate by radical-induced reduction in the presence of tributyltin hydride and AIBN gave 3-deoxy thioglycoside **84** in 33 % yield after two steps. The subsequent glycosidation of **84** with acceptor **65** using NIS and TfOH

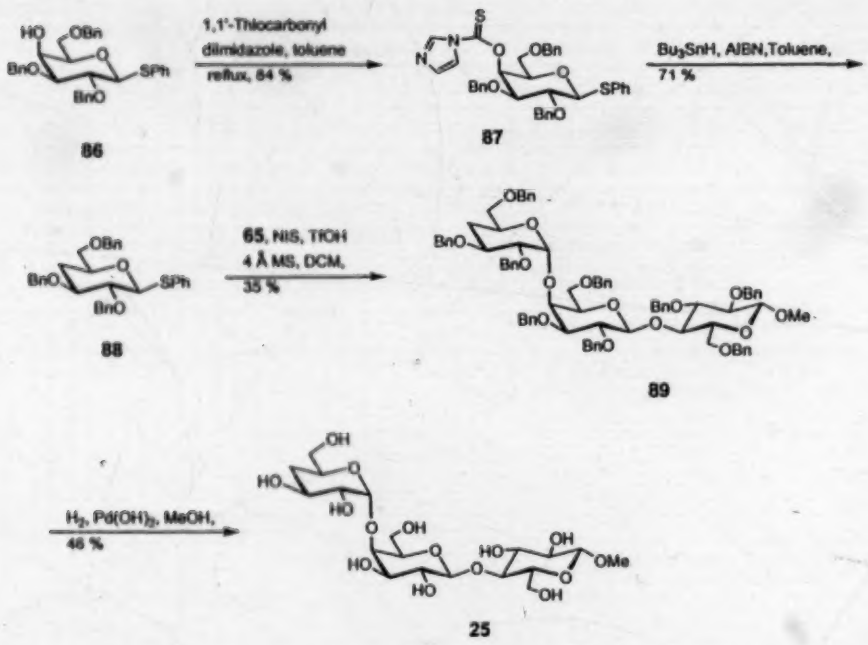
as promoters yielded the trisaccharide **78** in 49 % yield. Deprotection of **78** using hydrogen and palladium hydroxide afforded the target 3″-deoxy P^k-trisaccharide **24** in 93 % yield.

2.3.4d Synthsis of 4″-deoxy analog



Scheme 2.18 *Synthesis of derivative* ***86***

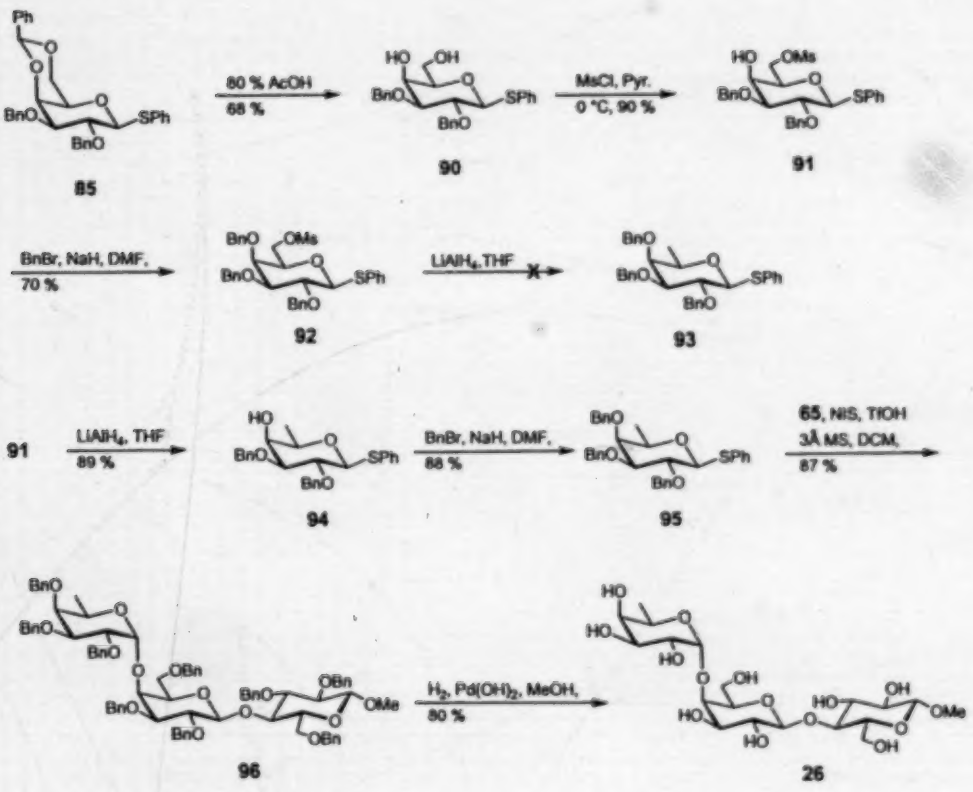
The 4″-deoxy analog **25** was synthesized using the same reaction sequence that was employed to prepare the 2″- and 3″-deoxy analogs. Phenyl thiogalactoside **69** was obtained from peracetylated galactose **67** in 75 % yield. Subsequent installation of a 4, 6-*O*-benzylidene acetal and benzylation of the remaining positions afforded the fully protected derivative **85** in 81% yield after 2 steps. Regioselective opening of the benzylidene acetal using sodium cyanoborohydride yielded alcohol **86** in 73 % yield.



Scheme 2.19 *Synthesis of 4"-deoxy P^k-trisaccharide analog 25*

The 4-deoxy galactose derivative **88** was obtained from compound **86** in 60 % yield after 2 steps employing Barton-McCombie deoxygenation methodology. The deoxygenated derivative was then glycosidated with acceptor **65** using NIS and TfOH to obtain the fully protected trisaccharide **89** in 35 % yield. The hydrogenolysis of **89** using palladium hydroxide as a catalyst afforded the targeted 4″-deoxy P^k-trisaccharide **25** in 46 % yield.

2.3.4e Synthesis of 6″-deoxy analog



Scheme 2.20 *Synthesis of 6″-deoxy P^{k}-trisaccharide analog* ***26***

The fully protected derivative **85** was synthesized in three steps as previously described (Scheme 2.18). Hydrolysis of benzylidene acetal **85** using 80 % acetic acid gave diol **90** in 68 % yield. The mesylation at the 6-*O*-position of **90** followed by benzylation afforded compound **91** in 63 % yield after two steps. Attempts to obtain the 6-deoxy derivative **93** by reduction of the mesyl group of **92** with lithium aluminum hydride failed. It was then envisaged that the 6-deoxy compound could be

obtained by using a derivative that is sterically less hindered at the C-6 position. Indeed, reductive deoxygenation of compound **91** with lithium aluminium hydride produced alcohol **94** in 89 % yield. The hydroxyl group of **94** was benzylated using benzyl bromide to afford donor **95** in 88 % yield. The glycosidation of **95** with **65** using NIS and TfOH as promoters gave trisaccharide **96** in 87 % yield. Total deprotection of **96** provided target compound **26** in 80 % yield.

Chapter 3

Analysis of the Binding Affinity of Synthetic Carbohydrate Receptors to Shiga toxin

3.1 Background

Isothermal titration microcalorimetry (ITC),[139] surface plasmon resonance (SPR),[140] and frontal affinity chromatography-mass spectrometry (FAC/MS)[141] are quantitative techniques for evaluating ligand-protein binding and are especially well suited to study sugar-protein interactions. Each of the techniques exhibits strong and weak points. ITC provides a direct measure of the enthalpy of association and provides direct information on binding stoichiometry. However, it needs large amounts of both protein and carbohydrate (milligram quantities) for each analysis. SPR is sensitive technique that measures the rate constant of the association-dissociation reaction. SPR and FAC/MS have a similar limitation, which is the requirement for the immobilization of the analytes on a solid surface.

Mass spectrometry (MS), using a soft ionization technique, is a powerful tool for studying noncovalent biomolecular complexes.[142] Of the available ionization techniques, electrospray (ES) and nanoflow electrospray (nanoES) have proven to be the most useful for investigating interactions between soluble, biologically relevant molecules.[143] ES and nanoES-MS with its speed and sensitivity has tremendous potential for quantifying binding affinities of biomolecular complexes, including protein-protein,[144] protein-peptide,[145] protein-oligonucleotide,[146] RNA-binding antibiotics[147] and small molecule-RNA complexes.[148] The interaction between the P^k

trisaccharide and the B_5 subunit of Stx1 was the first example of quantitative evaluation of protein-carbohydrate binding by FAC/MS.[149]

The mass-spectrum of the protein-carbohydrate mixture is usually very complex. It contains the starting compound and several complexes, all of which exist in the form of several charge states. To analyze the spectrum a binding model should be implemented in each particular case. For example, to describe the binding of a ligand (L) to the 5 independent binding sites of a pentavalent protein (P) the following reactions must be considered:

$$P + L \rightleftharpoons P \cdot L \qquad (5a)$$

$$P \cdot L + L \rightleftharpoons P \cdot L_2 \qquad (5b)$$

$$P \cdot L_2 + L \rightleftharpoons P \cdot L_3 \qquad (5c)$$

$$P \cdot L_3 + L \rightleftharpoons P \cdot L_4 \qquad (5d)$$

$$P \cdot L_4 + L \rightleftharpoons P \cdot L_5 \qquad (5e)$$

Equilibrium concentrations of bound protein are determined from the relative abundance of the corresponding ions observed in the mass spectrum according to equation 6b.

$$[P]_o = [P]_{equil} + [PL]_{equil} + [PL_2]_{equil} + [PL_3]_{equil} + [PL_4]_{equil} + [PL_5]_{equil} \qquad (6a)$$

$$[L]_o = [L]_{equil} + [PL]_{equil} + 2[PL_2]_{equil} + 3[PL_3]_{equil} + 4[PL_4]_{equil} + 5[PL_5]_{equil} \qquad (6b)$$

For equivalent binding sites, the following general expression allows K_{assoc} to be determined for each and any of the ligand binding reactions described by equation 5 where p is the number of occupied binding sites[150]:

$$K_{assoc,\,p} = K_{assoc}(5 - p + 1)/p \qquad (7)$$

An average K_{assoc} can be determined from the binding constant determined for each of the binding reactions, equations 8a-8e.

$$[PL]/[P][L] = 5K_{assoc} \qquad (8a)$$

$$[PL_2]/[PL][L] = 4K_{assoc}/2 \qquad (8b)$$

$$[PL_3]/[PL][L] = 3K_{assoc}/3 \qquad (8c)$$

$$[PL_4]/[PL][L] = 2K_{assoc}/4 \qquad (8d)$$

$$[PL_5]/[PL][L] = K_{assoc}/5 \qquad (8e)$$

where the numeric factors represent statistical coefficients according to different probabilities of the direct and reverse reaction.

Changes to the intensities measured for the ions of the unbound protein (P) and specific protein-ligand complexes (PL, PL_2, …, PL_N+) result from the occurrence of nonspecific protein-ligand binding during the nanoES process. The influence of nonspecific binding for the P^{n+} ion on the measured intensity (I_{app}) is quantitatively described by the following expression:

$$I_{app}(P^{n+}) = I(P^{n+}) - f_{1,P}I(P^{n+}) - f_{2,P}I(P^{n+}) - f_{3,P}I(P^{n+}) - \ldots - f_{i,P}I(P^{n+}) \qquad (9a)$$

where $I(P^{n+})$ is the ion intensity of P^{n+} expected in the absence of nonspecific binding and $f_{i,P}$ represents the fraction of the protein that is bound nonspecifically to i molecules of L. Given that $\sum f_{i,P} = 1$ (where i = 0, 1, 2, ...), equation 9a can be rewritten as

$$I(P^{n+}) = I_{app}(P^{n+})/f_{0,P} \qquad (9b)$$

where $f_{0,P}$ is the fraction of P that does not undergo nonspecific binding. The apparent intensity of a specific PL complex is likewise influenced by nonspecific binding according to the following expression:

$$I(PL^{n+}) = [I_{app}(PL^{n+}) - f_{1,P}I(P^{n+})]/f_{0,PL} \qquad (9c)$$

where $I(PL^{n+})$ is the expected ion intensity for the specific PL, $f_{1,P}$ is the fraction of P that binds one ligand nonspecifically, and $f_{0,PL}$ is the fraction of PL that does not undergo nonspecific binding.

The measured intensity for a protein bound specifically to five ligands (i.e., PL_5) is influenced by nonspecific binding according to the following expression:

$$I(PL_5^{n+}) = [I_{app}(PL_5^{n+}) - f_{1,PL4}I(PL_4^{n+}) - f_{2,PL3}I(PL_3^{n+}) - \ldots f_{5,P}I(P^{n+})]/f_{0,PL5} \qquad (9d)$$

The above expression allows for the influence of nonspecific ligand binding on the measured ion intensities for unbound protein and specific protein-ligand complexes to be accounted for quantitatively. Because the corresponding f_i terms must be known, we used the approach described in one article.[151] An appropriate reference protein (P_{ref}) is selected which does not bind specifically to any solution components. The occurrence of nonspecific protein-ligand binding is indicated by nonspecific complexes (P_{ref} + ligand) in the mass spectrum. The contribution of nonspecific binding to the measured intensities of protein and specific protein-ligand complexes can be quantitatively assessed based on the fraction of P_{ref} undergoing nonspecific ligand binding. Errors introduced into the protein-ligand association constant by nonspecific ligand binding are effectively corrected by this method.

3.2 FT-ICR MS Analysis of Deoxy P^k-trisaccharides

Stx1 B was expressed in *E. coli* using a procedure reported previously.[152] Stx1 B used in this work was generously provided by Glen Armstrong (University of Calgary). Proteins were purified to more than 95% purity by affinity chromatography, as reported;[153] dialyzed against 50 or 100 mM of ammonium acetate (pH 7); and stored at -20 °C. A stock solution of the Stx1 B subunit at a concentration of 1.15 mg/ml (150 μM) in 50 mM of ammonium acetate was prepared. The nanoES solutions were prepared by thawing the stock solutions at room temperature and diluting an aliquot to a concentration of 5 μM with aqueous ammonium acetate.

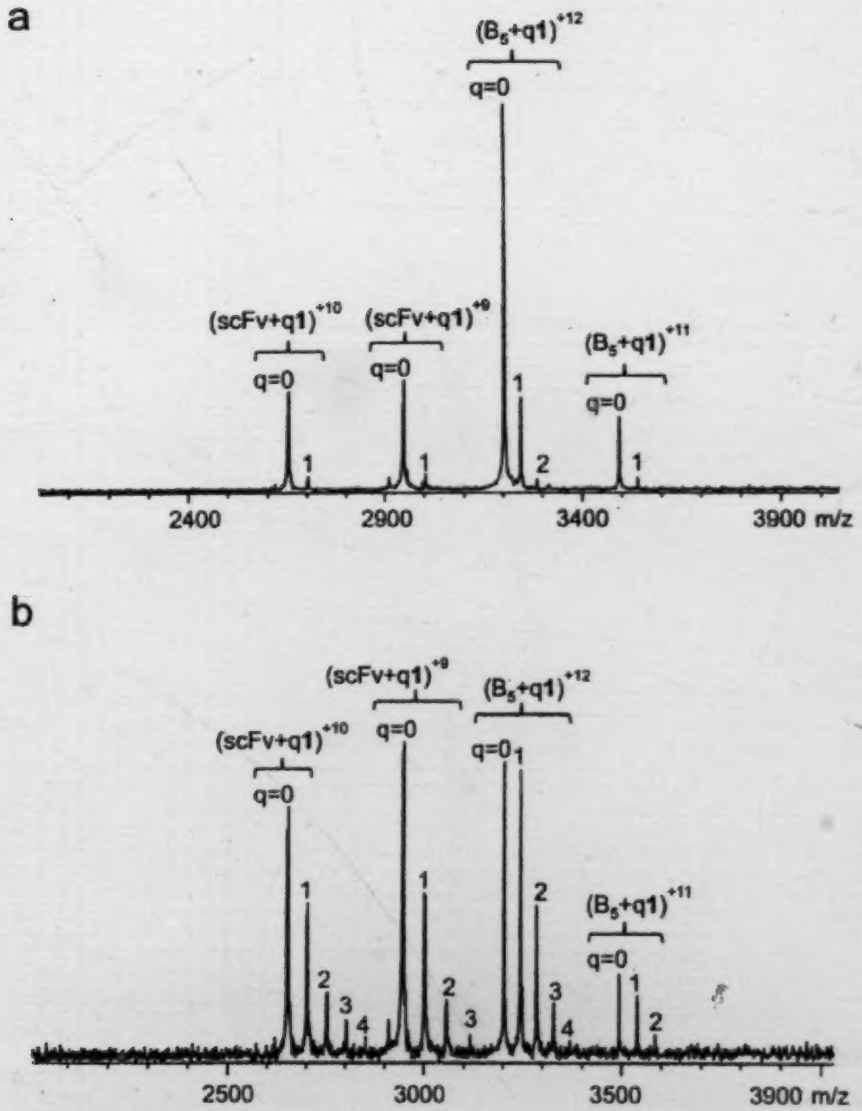


Figure 3.1 *NanoES-MS spectrum of a solution consisting of 10 mM ammonium acetate (pH 7), 7 μM Stx1 B_5 and* **26** *(6"-deoxy analog) at concentration of (a) 60 μM and (b) 120 μM. A reference protein, scFv, was added to both solutions at a concentration of 5 μM to quantify the extent of nonspecific protein-ligand during the ES process.*

I would like to acknowledge Dr. John Klassen and Dr. Elena Kitova for helping me to get the nanoES spectroscopic data. The structures of the ligands are shown in Figure 2.1. Prior to weighing samples, any absorbed water was removed from the ligands prior to the preparation of stock solutions by drying the ligands in a vaccum chamber maintained at ~5 torr and 56 °C. Stock ligand solutions were prepared by dissolving 1 mg in water at concentration 10^{-3} M and stored at -20 °C.

Single chain anibody Se15S-4 was used as a P_{ref} in the current work, nanoES spectra of solutions Stx1 B and P^k or P^k-based 6″-deoxy ligand (**26**) are shown in Figure 3.1. NanoES mass spectra were measured for equimolar solutions of Stx1 B_5 and scFv (5μM) and **26** over a concentration range from 60 to 200 μM in order to determine the K_{assoc} values for the binding of **26** with Stx1 B_5. A mass spectrum obtained for a solution containing 60 μM of **26** is shown in Figure 3.1a. The B_5 homopentamer is observed B_5^{n+}, where n = 11, 12, predominantly in its unbound form, $(B_5+\mathbf{26})^{n+}$ and $(B_5+2(\mathbf{26}))^{n+}$, only a small fraction bound of a single molecule of **26**. The $scFv^{n+}$ ions are observed in their unbound form, with no detectable $(scFv+\mathbf{26})^{n+}$ ions. The spectrum shown in Figure 3.1b was acquired for the solution with higher concentration of 26 (120 μM). B_5^{n+}, $(B_5+\mathbf{26})^{n+}$, $(B_5+2(\mathbf{26}))^{n+}$, $(B_5+3(\mathbf{26}))^{n+}$, and $(B_5+4(\mathbf{26}))^{n+}$ ions were observed, along with ions corresponding to $scFv^{n+}$, $(scFv+\mathbf{26})^{n+}$, $(scFv+2(\mathbf{26}))^{n+}$, $(scFv+3(\mathbf{26}))^{n+}$, and $(scFv+4(\mathbf{26}))^{n+}$.

From the relative peak intensities corresponding to bound and unbound proteins, the average K_{assoc} values were determined using eq. 9d. All results are shown in Table 3.1.

	K_{assoc}, M^{-1}	Stdev K_{assoc}	-ΔG (kcal/mol)	ΔΔG (kcal/mol)
Native P^k-OMe (**11**)	358	10	3.48	0.0
2′-deoxy (**20**)	255	5	3.28	0.2
3′-deoxy (**21**)	305	6	3.39	0.1
6′-deoxy (**22**)	135	29	2.90	0.6
2″-deoxy (**23**)	194	9	3.12	0.4
3″-deoxy (**24**)	10		1.36	2.1
4″-deoxy (**25**)	185	2	3.09	0.4
6″-deoxy (**26**)	331	6	3.44	0.0

Table 3.1 *K_{assoc} values determined by nanoES for binding of Stx1 B with native P^k-OMe (11) and its deoxy analogs (20-26) at 298 K.*

Based on calorimetry measurements, the interaction of the B subunit of Stx 1 with **11** (Figure 2.1) gave a K_{assoc} value of (0.5-1) x 10^3 M^{-1} per monomer (site 2) and a ΔG value of -3.6 kcal mol^{-1}.[154] As a result, the K_{assoc} value of **11**, as revealed by nanoES-MS, is in close agreement with theses values. However, the Stx1-P^k trisaccharide binding constant is weaker than most typical protein-carbohydrate binding constants (range of 10^3-10^6 M^{-1}). The calorimetry measurement also has shown that binding of **11** to B_5 is noncooperative.

The thermodynamic binding data for native P^k-OMe **11** and deoxy analogs (**20-26**) are shown in Table 3.1. The binding parameters of **26** (6″-deoxy) are nearly the same as that of **11**, indicating no binding affinity of the 6″-OH group. The K_{assoc} value of **20** (2′-deoxy), **21** (3′-deoxy) and **26** (6″-deoxy) did not show significant difference to native P^k-OMe **11**. On the other hand, the K_{assoc} value of **24** (3″-deoxy) is significantly lower than that of **11**, indicating that the 3″-OH group in the P^k trisaccharide is primarily responsible for their high affinity binding to Stx1 B.

Chapter 4

Conclusions

We have successfully synthesized the P^k-trisaccharide, which is a ligand for Shiga toxins, and seven P^k-trisaccharide analogs (**11**, **20**, **21**, **22**, **23**, **24**, **25** and **26**) containing the galabiose moiety (α-Gal*p*-(1→4)-β-Gal*p*) deoxygenated at either the 2′, 3′, 6′, 2″, 3″, 4″, or 6″ positions. These compounds were then used to evaluate their binding to the Stx1 B subunit by FTICR-MS. With regard to efficiency, the synthetic strategies we used compare well with syntheses of similar deoxy P^k-trisccharide analogs reported by other groups.[137] Although our strategies were novel, the methods we employed to build key linkages in our synthesis were derived from known methods in carbohydrate synthesis. Thioglycoside and trichloroacetimidate donors proved to be donors of choice, whereas, the Koenigs-Knorr method with glycosyl halides as donors was also implemented for the formation of some glycosidic bonds. With the exception of the 2′-deoxy analog **20**, in which the 1,4-linkage was constructed chemo-enzymatically, all of the (1→4)-glycosidic linkages were synthesized employing chemical methods. Deoxy analogs **21**, **24** and **25** were synthesized using the Barton-McCombie deoxygenation method.

The recently developed FTICR-MS method[144-149] was used to obtain affinity constants for the binding of the synthesized ligands to Stx1 B. The results show that all analogs bind to the subunit of Stx1 and their affinities can be estimated. With the exception of the 3″-deoxy analog, all deoxy derivatives exhibit similar activities to the native P^k-trisaccharide methyl glycoside. A more detailed comparison shows that

binding measurements are accompanied by errors introduced by non-specific binding of ligands to the protein due to the high concentrations of ligands that had to be used for the measurement of binding constants of such low magnitude. However, corrections for this electrospray-induced non-specific binding were made.

In order to fully understand the interactions of proteins and carbohydrates two types of information, structural and energetic, are required. Carbohydrate-protein interactions are generally weak. Frequently, the values of ΔH and ΔS are large and negative; consequently the resultant free energy remains small. One of the best examples for this is the interaction between the B subunit of Stx1 and P^k-trisaccharide. The binding energy for interaction between the B subunit of Stx1 and native P^k-trisaccharide methyl glycoside **11** was estimated to be -3.5 kcal/mol, which is in agreement with a ΔG value of -3.6 kcal/mol obtained by microcalorimetry measurement as previously reported.[154] The success in measuring such small association constants and free energies rests on the large exothermic enthalpy changes for this binding event. Entropy can be calculated using ΔH and K_a, which were measured by microcalorimetry. The technique needs large amounts of both protein and carbohydrate and large ΔH change. Despite a low binding energy (ΔG = -3.6 kcal/mol), the enthalpy of binding is strongly exothermic (ΔH = -12 kcal/mol) and entropic component ($T\Delta S$ = -8.4 kcal/mol) is unfavorable. ΔH and ΔS can also be obtained indirectly from a van't Hoff plot of the temperature dependence of the binding constant, measured by a variety of techniques.

The conformation of the oligosaccharide in the bound state also influences the free energy of binding between carbohydrate and proteins. In solution,

oligosaccharides populate numerous conformational micro-states. Every hydroxyl group can sample numerous lower energy states for rotation about each carbon oxygen bond. Similarly the hydroxymethyl group also samples at least the three staggered conformers about the C5-C6 bond of every hexopyranose residue. Conformations about the glycosidic bond defined as H1-C1-O1-Cx and about the aglyconic bond defined as C1-O1-Cx-Hx are of greater significance to the gross topology of any oligosaccharide. The torsional angle defined as H1-C1-O1-Cx generally adopts angles that fall within a narrow absolute range of 40-55°. The aglyconic torsional angle C1-O1-Cx-Hx may adopt a wider range of values but these often fall in the rangle +30° to -30°. When the angles adopted fall within this range a stronger NOE exists between the anomeric hydrogen and the hydrogen attached to the aglyconic carbon atom. This NOE can be observed almost without exception in any oligosaccharide. In the bound state oligosaccharides generally adopt conformations close to low energy conformations that are heavily populated in the unbound state. A limited number of exceptions to these general conclusions have been reported for enzyme-inhibitor complexes and for oligosaccharides bound to antibodies, although in the latter case the glycosidic bond in question was at the periphery of the site. The anti-conformation observed was imposed by the conformation of those residues that were most buried in the site.

So, while low energy conformations of oligosaccharides are most likely to be observed in the bound state, one must consider an oligosaccharide as a relatively flexible molecule. When a flexible molecule binds it not only loses translational freedom but also conformational freedom due to the adoption of a relatively rigid

bond conformation. This results in a positive and unfavourable entropy contribution to ligand binding. Formation of strong hydrogen bonds will impose further restraints on the rotation of hydroxyl groups. The hydrogen bonds provide the exothermic contributions to enthalpy while the loss of motional freedom for the oligosaccharide and amino acid side chains that engage it account for substantial parts of the unfavourable entropy. The role of water is complex and can provide favourable and unfavourable contributions to enthalpy and entropy.

The crystal structure of Stx with P^k trisaccharide indicates many intermolecular hydrogen bonding, total of 11 hydrogen bonds at site 2. The average ΔH value is 1.1 kcal/mol per hydrogen bond. The strength of a hydrogen bond can be estimated based on heavy atom distances. In this ligand the crystal structure data is consistent with previous binding data for Gb_3 congeners. In the crystal structure the P^k analog contains a single alkyl chain as glycolipid. However, our inhibitors have no lipid moiety, which may affect the precise mode of binding. Because the aglycon is small minor adjustments in the position of the trisaccharide may occur in the bound state and the conformation of the saccharide relative to the lipid may be influential. Water mediated hydrogen bonding interactions may be important in binding, but at the relatively low resolution (2.8 Å) the position of water molecules can not be defined with precision.

Within experimental error, the activities of the 2′-deoxy **20**, 3′-deoxy **21** and 6″-deoxy **26** are not significantly different from that of native P^k-trisaccharide **11**. However, the K_{assoc} value of 3″-deoxy **24** is substantially lower than that of native P^k-

trisaccharide 11. Based on the thin layer chromatography (TLC) overlay assay reported by Lingwood and co-workers for deoxy Gb_3 glycolipid analogs,[155] the

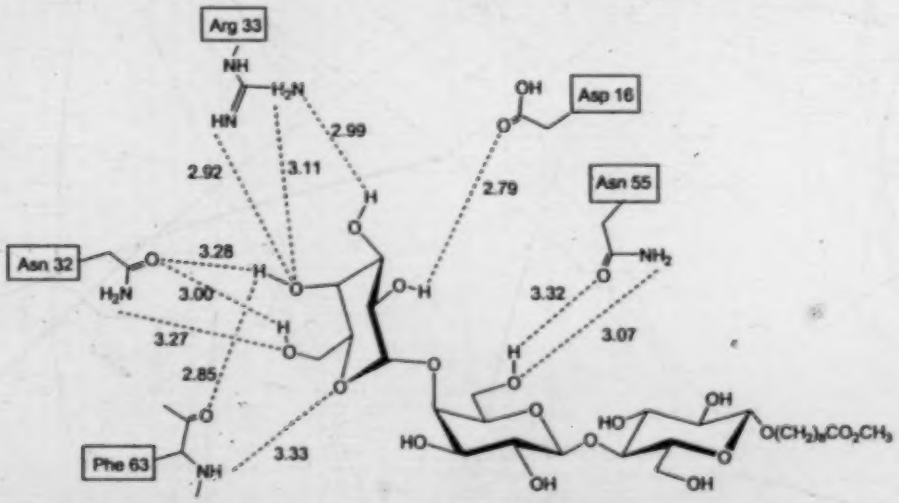


Figure 4.1 *Hydrogen bonding map for the Stx1B-P^k-MCO complex at site 2*

contribution of each hydroxyl group in P^k-trisaccharide decreases in the following order: 6″≈6′ > 2′ > 4″ > 2″ > 3′≈3″ for Stx1. In contrast, our binding studies performed in solution using a panel of water soluble deoxy analogs of P^k-trisaccharide described in this thesis indicate that the hydrogen bond provided by the 3″-OH group in P^k-trisaccharide is critically important for the binding to Stx 1 and Stx 2. This apparent contradiction may be attributed to the kinetic nature of the TLC overlay assay, which discriminates binding sites according to their rate of interaction, whereas, FTICR assay measures the affinity of the ligand for the most dominant binding sites under equilibrium conditions. A complicating factor may also be the presence of the sphingolipid attached to the oligosaccharide in the perspective of ligand toxin interactions TLC assay.

In addition to providing new data, our binding studies employing nanoES-MS indicate that binding constants of the order of 1 mM^{-1} represent the lower limit of the method. Utilizing of ligands with lower affinities for the purpose of binding site mapping will inevitably pose problems for confident evaluation of affinities.

Chapter 5

Experimental

5.1. General Methods

5.1.1 Reagent and Chromatography

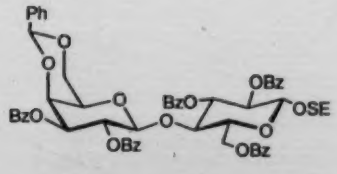
All reagents were used as supplied from commercial sources. Solvents were distilled and dried from SPS-400-7 containing appropriate drying reagent. Analytical thin layer chromatography (TLC) was performed on silica gel 60-F_{254} from Merck. TLC detection was achieved by using UV light and 5% sulfuric acid in ethanol or acidic cerium ammonium molybdate followed by heating. Column chromatography was conducted with silica gel 60 (230-40 mesh) from SiliCycle. High-performance liquid chromatography utilized a Waters Delta 600 system using an absorbance detector. Separation was done using a C18-silica semi-preparative reverse phase column with an increasing gradient of methanol in water. Solid-phase extraction cartridges (Sep-Pak, C-18) were purchased from Waters Corp. (Milford, MA, USA).

5.1.2 Spectral Analysis

^{1}H NMR spectra were recorded on Varian INOVA 500 or 600 MHz spectrometers and ^{13}C NMR spectra were recorded at 125 MHz. All spectra were recorded at 27 °C. ^{1}H NMR spectra were referenced to residual proton solvent signals and reported in ppm (δ = 7.24 for $CDCl_3$, 3.30 for CD_3OD). ^{13}C NMR spectra were referenced to the solvent resonance and reported in ppm (δ = 77.0 for $CDCl_3$, 49.0 for

CD_3OD). Most of the ^{13}C NMR data were obtained from APT experiments performed on a 500 MHz spectrometer. Mass spectrometry analysis was performed using positive mode electrospray ionization on a Zabspec hybrid Sectore-TOF instrument.

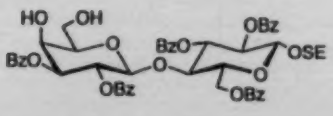
5.1.3 Synthesis



2-(Trimethylsilyl)-ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galacto pyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (31).

2-(Trimethylsilyl) ethyl lactoside **30**[124] (4.75 g, 10.7 mmol) was dissolved in dry MeCN (40 ml). Benzaldehyde dimethylacetal (3.23 ml, 21.6 mmol) and *p*-TsOH (185 mg) were added and the mixture was stirred for 1 h. The solution was neutralized with triethylamine. The reaction mixture was then concentrated to dryness to give a white solid to which dry pyridine (11.8 ml, 0.2 mol) was added followed by benzoyl chloride (17.5 ml, 0.15 mol). After stirring for 10 minutes the reaction mixture was diluted with DCM and the organic phase washed with 5% HCl, saturated aqueous sodium bicarbonate and brine. Evaporation of the organic solvent gave a light yellow residue that was purified by column chromatography (1:4 EtOAc:hexane) to give 4.75 g (42 %, 2 steps) of **31**: $[\alpha]_D$ +101.0 (c = 0.2, $CHCl_3$). ^1H NMR (600 MHz,

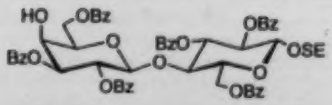
$CDCl_3$) δ 8.12-7.17 (m, 25H, Ar), 5.84-5.77 (m, 2H, H-2′, H-3), 5.33-5.29 (m, 2H, H-2, PhC<u>H</u>O_2), 5.17 (dd, 1H, J = 10.2, 3.6 Hz, H-3′), 4.85 (d, 1H, J = 7.8 Hz, H-1′), 4.70 (d, 1H, J = 7.8, H-1), 4.62 (dd, 1H, J_{gem} = 13.8 Hz, $J_{5,6}$ = 1.8 Hz, H-6a), 4.38 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 4.8 Hz, H-6b), 4.31 (d, 1H, J = 3.6 Hz, H-4′), 4.21 (dd, 1H, J = 9.0 Hz, H-4), 3.89-3.84 (m, 2H, H-5, O<u>CH_2</u>CH_2), 3.77 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5';6'}$ = 1.2 Hz, H-6′a), 3.58 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5';6'}$ = 1.8 Hz, H-6′b), 3.50 (m, 1H, O<u>CH_2</u>CH_2), 2.98 (dd, 1H, $J_{5';6'a}$ = 1.2 Hz, $J_{5';6'b}$ = 1.8 Hz, H-5′), 0.85-0.75 (m, 2H, OCH_2<u>CH_2</u>), -0.13- -0.25 (m, 9H, $SiCH_3$). ES HRMS Calcd. for $C_{59}H_{58}O_{16}SiNa$ (M+Na):1073.3391. Found 1073.3390.



2-(Trimethylsilyl)-ethyl 2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (32).

The benzylidene acetal **31** (1.178 g, 1.12 mmol) was suspended in methanol (10 ml) and DCM (10 ml). Palladium hydroxide was added and the reaction stirred under a hydrogen atmosphere for 24 h. The reaction mixture was filtered through a Millipore millex-LCR cartridge. The organic solvent was evaporated and column chromatography in EtOAc:toluene (1:4) gave a colorless syrup (1.042 g, 97 %): $[\alpha]_D$ +64.0 (c = 0.2, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02-7.20 (m, 25 H, Ar), 5.75-5.71 (m, 2H, H-2′, H-3), 5.39 (dd, 1H, J = 9.5, 7.5 Hz, H-2), 5.07 (dd, 1H, J = 10.0,

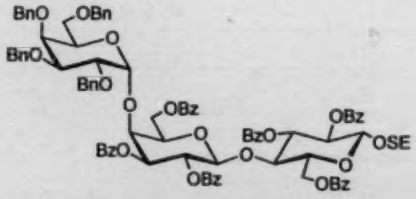
3.0 Hz, H-3′), 4.78 (d, 1H, J = 8.0 Hz, H-1′), 4.70 (d, 1H, J = 8.0 Hz, H-1), 4.60 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 2.0 Hz, H-6a), 4.41 (dd, 1H, J_{gem} = 14.5 Hz, $J_{5,6}$ = 5.0 Hz, H-6b), 4.19-4.15 (m, 2H, H-4, H-4′), 3.91-3.84 (m, 2H, H-5, O$\underline{CH_2}CH_2$), 3.55-3.50 (m, 1H, O$\underline{CH_2}CH_2$), 3.37-3.35 (m, 2H, H-5′, H-6′a), 3.27 (dd, 1H, J_{gem} = 13.0 Hz, $J_{5',6'}$ = 6.0 Hz, H-6′b), 0.84 (m, 2H, OCH$_2\underline{CH_2}$), 0.14-0.15 (m, 9H, SiCH$_3$). ^{13}C NMR (125 MHz) δ 165.8, 165.7, 165.5, 165.2, 165.0, 134.5, 133.5, 133.4, 133.3, 133.1, 130.1, 129.8, 129.7, 129.6, 129.5, 129.4, 129.0, 128.8, 128.6, 128.4, 128.3, 128.2, 101.3, 100.1, 76.7, 74.3, 74.2, 73.7, 72.8, 71.9, 69.8, 67.9, 67.4, 62.8, 62.2, 17.8, -1.4, -1.5. Anal. Calcd. for $C_{52}H_{54}O_{16}Si$: C, 64.85; H, 5.65. Found: C, 64.85; H, 5.69. ES HRMS Calcd. for $C_{52}H_{54}O_{16}SiNa$ (M+Na):985.3073. Found 985.3072.



2-(Trimethylsilyl)-ethyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (33).

Diol **32** (1.042 g, 1.08 mmol) was dissolved in dry pyridine (10 ml), and a catalytic amount of DMAP was added. The solution was cooled to 0 °C and benzoyl chloride (0.15 ml, 1.29 mmol) was added. After stirring for 10 min the reaction mixture was diluted with DCM and the organic phase was washed with 5% HCl, saturated aqueous sodium bicarbonate and brine. Evaporation of the organic solvent gave a light yellow residue that was purified by column chromatography (1:19 EtOAc:toluene) to get

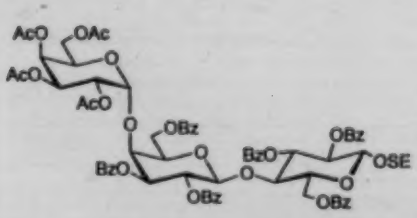
866 mg (75 %) of compound **33**: $[\alpha]_D$ +54.5 (c = 0.6, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 8.03-7.15 (m, 30H, Ar), 5.76 (t, 1H, *J* = 9.6 Hz, H-3), 5.72 (dd, 1H, *J* = 10.5, 8.7 Hz, H-2'), 5.43 (dd, 1H, *J* = 10.2, 8.4 Hz, H-2), 5.15 (dd, 1H, *J* = 10.2, 3.0 Hz, H-3'), 4.78 (d, 1H, *J* = 7.8 Hz, H-1'), 4.71 (d, 1H, *J* = 8.4 Hz, H-1), 4.59 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 1.8 Hz, H-6a), 4.47 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 5.4 Hz, H-6b), 4.21 (t, 1H, *J* = 9.6 Hz, H-4), 4.14-4.09 (m, 2H, H-4', H-5'), 3.93-3.92 (m, 1H, $O\underline{CH_2}CH_2$), 3.86-3.83 (m, 1H, H-5), 3.68-3.63 (m, 2H, H-6'a/b), 3.56-3.51 (m, 1H, $O\underline{CH_2}CH_2$), 0.91-0.79 (m, 2H, $OCH_2\underline{CH_2}$), 0.11-0.12 (m, 9H, $SiCH_3$). ^{13}C NMR (125 MHz) δ 166.0, 165.8, 165.7, 165.6, 165.2, 164.9, 133.4, 133.2, 133.1, 133.0, 129.8, 129.7, 129.6, 129.5, 128.9, 128.8, 128.6, 128.4, 128.3, 128.2, 101.0, 100.3, 76.3, 74.1, 73.2, 72.9, 72.6, 71.9, 69.7, 67.4, 66.8, 62.8, -1.5. Anal. Calcd. for $C_{59}H_{58}O_{17}Si$: C, 66.40; H, 5.48. Found: C, 66.20; H, 5.49. ES HRMS Calcd. For $C_{59}H_{58}O_{17}SiNa$ (M+Na):1089.3335. Found 1089.3338.



2-(Trimethylsilyl)-ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (35).

The selectively protected lactoside **33** (0.18 g, 0.158mmol), thioglycoside donor **34** (0.144 g, 0.22 mmol), and crushed 3 Å MS (1 g) was stirred in dry DCM (10 ml) at room temperature under argon for 1 h. The mixture was treated with *N*-iodosuccinimide (50 mg, 0.22 mmol) and trifluoromethanesulfonic acid (3 µl, 0.033 mmol). After 1h 30 min the mixture was diluted with DCM, filtered through Celite, and the organic layer was washed sequentially with saturated sodium thiosulfate, saturated sodium bicarbonate, water, and brine. The organic phase was dried over sodium sulfate and concentrated under reduced pressure. Column chromatography of the residue using hexane:EtOAc (4:1) gave **35** (0.165 g, 70 %) as a colorless form: $[\alpha]_D$ +48.9 (c = 0.3, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03-7.07 (m, 50H, Ar), 5.80 (t, 1H, J = 9.0 Hz, H-3), 5.75 (dd, 1H, J = 9.6, 7.8 Hz, H-2′), 5.37 (dd, 1H, J = 9.0, 8.4 Hz, H-2), 5.05 (dd, 1H, J = 10.8, 2.4 Hz, H-3′), 4.89 (d, 1H, J = 6.6 Hz, H-1′), 4.82 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.75-4.66 (m, 5H, H-1, H-1″, H-6a, $PhCH_2$), 4.58-4.55 (m, 3H, H-6′a, $PhCH_2$), 4.59 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 3.6 Hz, H-6b), 4.46 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.31 (s, 1H, H-4′), 4.27-4.23 (m, 3H, H-4, H-5″, $PhCH_2$), 4.20-4.17 (m, 2H, H-6′b, $PhCH_2$), 4.08 (d, 1H, J = 1.8 Hz, H-4″), 4.07-3.87 (m, 4H, H-5, H-2″, H-3″, $O\underline{CH_2}CH_2$), 3.69 (t, 1H, J = 6.6 Hz, H-5′), 353-3.49 (m, 1H, $O\underline{CH_2}CH_2$), 3.36 (t, 1H, $J_{gem} \approx J_{5'',6''}$ = 9.0 Hz, H-6″a), 3.01 (dd, 1H, J_{gem} = 7.2 Hz, $J_{5'',6''}$ = 4.8 Hz, H-6″b), 0.89-0.78 (m, 2H, $OCH_2\underline{CH_2}$), 0.12 (s, 9H, $SiCH_3$). ^{13}C NMR (125 MHz) δ 166.4, 165.8, 165.6, 165.3, 165.2, 165.0, 138.9, 138.5, 138.2, 133.1, 133.0, 132.9, 129.8, 129.7, 129.6, 129.0, 128.9, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 127.4, 127.3, 127.2, 101.2, 101.1, 100.2, 79.1, 76.7, 76.5, 75.8, 75.5, 74.9, 74.7, 74.4, 73.4, 73.3, 73.1, 73.0, 72.9, 72.6, 72.4, 69.9, 69.7, 67.4, 67.3, 62.6,

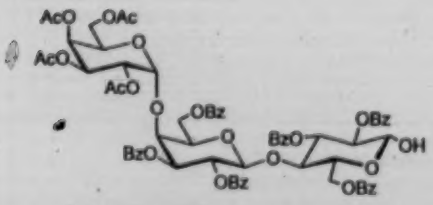
62.1, 17.8, -1.5. ES HRMS Calcd. for $C_{93}H_{92}O_{22}SiNa$ (M+Na):1611.5747. Found 1611.5750.



2-(Trimethylsilyl)-ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (36).

Trisaccharide **35** (0.165 g, 0.1 mmol) was suspended in dry methanol (10 ml). Palladium hydroxide on charcoal (10 % wt) was added and the solution stirred under a hydrogen atmosphere (balloon) for 6 h. The reaction mixture was filtered. The filtrate was concentrated to dryness to give a white solid to which dry pyridine (5 ml) was added followed by acetic anhydride (59 μl). The solution was stirred for 4 h. The solvent was evaporated, and the residue was purified by column chromatography using EtOAc:toluene (1:9) to give a white solid (0.107 mg, 73 %): $[\alpha]_D$ +83.7 (c = 0.12, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 8.04-7.14 (m, 30H, Ar), 5.78 (t, 1H, J = 9.0 Hz, H-3), 5.69 (dd, 1H, J = 10.8, 7.8 Hz, H-2′), 5.47 (d, 1H, J = 2.4 Hz, H-1″), 5.39 (dd, 1H, J = 9.0, 7.8 Hz, H-2), 5.33 (dd, 1H, J = 11.4, 3.6 Hz, H-2″), 5.15-5.11 (m, 2H, H-3, H-3″), 5.06 (d, 1H, J = 3.6 Hz, H-4″), 4.86 (d, 1H, J = 7.8 Hz, H-1′), 4.69 (d, 1H, J = 7.8 Hz, H-1), 4.62 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 2.4 Hz, H-6a), 4.49 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 5.4 Hz, H-6b), 4.45 (app t, 1H, J = 7.2 Hz, H-5″), 4.25 (d, 1H, J = 2.4 Hz, H-4′), 4.20 (t, 1H, J = 9.6 Hz, H-4), 4.01 (dd, 1H, J_{gem} = 11.4 Hz,

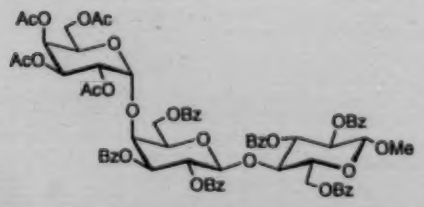
$J_{5',6'}$ = 7.2 Hz, H-6'a), 3.95 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5',6'}$ = 6.0 Hz, H-6'b), 3.90-3.86 (m, 2H, H-5, $O\underline{CH_2}CH_2$), 3.79 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5'',6''}$ = 7.8 Hz, H-6''a), 3.70-3.66 (m, 2H, H-5', H-6''b), 3.53-3.49 (m, 1H, $O\underline{CH_2}CH_2$), 2.05 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO), 1.12-0.77 (m, 2H, $OCH_2\underline{CH_2}$), 0.13 (s, 9H, $SiCH_3$). ^{13}C NMR (125 MHz) δ 170.4, 170.3, 170.0, 169.4, 166.1, 165.7, 165.4, 165.2, 165.0, 164.9, 133.6, 133.4, 133.2, 133.0, 129.7, 129.6, 129.5, 129.3, 128.6, 128.5, 128.4, 128.3, 128.2, 101.4, 100.2, 98.2, 75.2, 73.5, 73.3, 72.9, 72.6, 72.2, 69.5, 68.2, 67.7, 67.4, 67.1, 62.6, 61.3, 60.7, 20.6, 20.5, 17.8, -1.5. Anal. Calcd. for $C_{73}H_{76}O_{26}Si$: C, 62.74; H, 5.48. Found: C, 62.59; H, 5.45. ES HRMS Calcd. for $C_{73}H_{76}O_{26}SiNa$ (M+Na):1419.4291. Found 1419.4295.



2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (37).

Trisaccharide **36** (0.107 g, 0.077 mmol) was dissolved in DCM (0.5 ml), trifluoroacetic acid (1 ml) was added under argon and the mixture was stirred for 25 min. EtOAc (5 ml) and toluene (5 ml) were added and the solvents were removed under reduced pressure. A second portion of toluene (5 ml) was added and the volatiles were evaporated. Column chromatography of the residue using

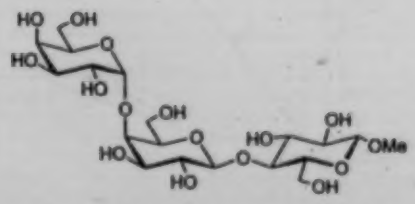
toluene:EtOAc (4:1) gave **37** (0.99 g, quant.) as a white solid: $[\alpha]_D$ +126.7 (c = 0.18, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 8.07-7.17 (m, 30H, Ar), 6.15 (t, 1H, *J* = 9.0, H-3), 5.72 (dd, 1H, *J* = 10.8, 7.8 Hz, H-2′), 5.58 (d, 1H, *J* = 3.6 Hz, H-1″), 5.44(d, 1H, *J* = 4.8 Hz, H-2), 5.34-5.31 (m, 1H, H-2″), 5.19-5.11 (m, 2H, H-3′, H-3″), 5.07-5.05 (m, 1H, H-4″), 4.93 (d, 1H, *J* = 7.8 Hz, H-1′), 4.87 (d, 1H, *J* = 7.8 Hz, H-1), 4.61-4.59 (m, 1H, H-6a), 4.53-4.49 (m, 1H, H-6b), 4.44-4.39 (m, 1H, H-5″), 4.26 (d, 1H, *J* = 8.4 Hz, H4′), 4.21 (t, 1H, *J* = 9.0 Hz, H-4), 4.07-3.99 (m, 2H, H-6′a/b), 3.79-3.60 (m, 4H, H-5, H-5′, H-6″a/b), 2.04 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.89 (s, 3H, CH_3CO). ^{13}C NMR (125 MHz) δ 170.4, 170.3, 170.0, 169.5, 166.9, 166.1, 165.9, 165.8, 165.4, 165.0, 164.9, 133.6, 133.5, 133.4, 133.3, 133.2, 133.1, 133.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 101.5, 101.4, 101.4, 98.2, 98.0, 95.6, 90.1, 75.0, 74.8, 74.5, 73.6, 73.5, 73.3, 72.6, 72.5, 72.4, 70.1, 69.6, 69.5, 68.4, 68.2, 67.7, 67.4, 67.1, 62.3, 61.2, 60.7, 20.6, 20.5. ES HRMS Calcd. for $C_{68}H_{64}O_{26}SiNa$ (M+Na):1319.3583. Found 1319.3584.



Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (38).

Derivative **37** (0.483 mg, 0.37 mmol) was dissolved in DCM (7 ml) and cooled to 0 °C. Trichloroacetonitrile (374 µl, 3.7 mmol) was added followed by DBU (6 µl, 0.04 mmol). The ice bath was removed, the reaction warmed to room temperature and stirred for 1 h. Concentration under reduced pressure gave the imidata as a dark brown syrup which was purified by column chromatography. The imidate (0.355 mg, 0.25 mmol) was dissolved in DCM (5 ml), 3 Å MS (500 mg) was added and the mixture was stirred for 30 min. Subsequently, methanol (1 ml) and borontrifluoride diethyletherate (22 µl, 0.18 mmol) were added and the reaction mixture was stirred overnight. After the addition of triethylamine the solution was filtered through Celite and washed with saturated sodium bicarbonate solution and water. The organic phase was concentrated under reduced pressure and the residue purified by column chromatography in EtOAc:toluene (1:4) to give a white solid (0.144 mg, 45 %): $[\alpha]_D$ +164.7 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05-7.06 (m, 30H, Ar), 5.80 (t, 1H, J = 9.6 Hz, H-3), 5.69 (dd, 1H, J = 10.8, 7.8 Hz, H-2′), 5.46 (d, 1H, J = 3.6 Hz, H- 1″), 5.38 (dd, 1H, J = 9.6, 7.8 Hz, H-2), 5.33 (dd, 1H, J = 10.8, 3.0 Hz, H-2″), 5.15-5.12 (m, 2H, H-3′, H-3″), 5.06 (d, 1H, J = 3.6 Hz, H-4″), 4.87 (d, 1H, J = 8.4 Hz, H-1′), 4.64 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 1.8 Hz, H-6a), 4.59 (d, 1H, J = 7.8 Hz, H-1), 4.50 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 4.2 Hz, H-6b), 4.44 (t, 1H, J = 6.9 Hz, H-5″), 4.25-4.21 (m, 2H, H-4, H-4′), 4.01 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5',6'}$ = 6.6 Hz, H-6′a), 3.96 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5',6'}$ = 6.6 Hz, H-6′b), 3.89-3.86 (m, 1H, H-5), 3.79 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5'',6''}$ = 7.8 Hz, H-6″a), 3.70-3.67 (m, 2H, H-5′, H-6″b), 3.41 (s, 3H, CH_3), 2.05 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 1.92 (s, 3H, CH_3CO). ^{13}C NMR (125 MHz) δ 170.4, 170.3, 170.0, 169.5, 166.1, 165.7, 165.4,

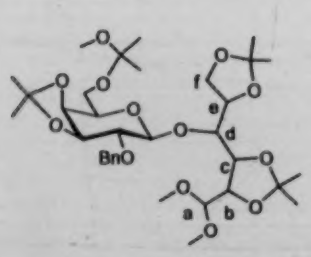
165.3, 165.0, 164.8, 133.6, 133.4, 133.2, 133.1, 129.8, 129.7, 129.6, 129.5, 129.3, 128.6, 128.5, 128.4, 128.3, 128.2, 101.6, 101.4, 98.2, 75.2, 73.5, 73.2, 73.0, 72.6, 72.1, 69.5, 68.2, 67.7, 67.4, 67.1, 62.4, 61.3, 60.7, 56.9, 20.6, 20.5. ES HRMS Calcd. for $C_{69}H_{66}O_{26}SiNa$ (M+Na):1333.3740. Found 1333.3739.



Methyl α-D-galactopyranosyl-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-gluco-pyranoside (11).

Trisaccharide methyl glycoside **38** (0.187 g, 0.14 mmol) was dissolved in methanol (3 ml), 0.5 M sodium methoxide in methanol (571 μl) was added under argon and the mixture was stirred for 3h. The solution was neutralized with Amberlite IR 120 resin and filtered. The reaction was purified using reverse-phase (C-18) HPLC (0-50 % MeOH). The product was collected and lyophilized to give a white powder (0.048 g, 65 %): $[\alpha]_D$ +179.2 (c = 0.1, H_2O). ^1H NMR (500 MHz, D_2O) δ 4.95 (d, 1H, J = 4.0 Hz, H-1″), 4.50 (d, 1H, J = 7.5 Hz, H-1′), 4.40 (d, 1H, J = 8.0, H-1), 4.35 (t, 1H, J = 7.0 Hz, H-5′), 4.04 (t, 1H, J = 3.5 Hz, H-4′), 4.01 (dd, J = 12.5, 2.5 Hz, H-3″), 3.94-3.89 (m, 2H, H-5″, H-6″a), 3.85-3.81 (m, 2H, H-2″, H-5), 3.78 (dd, 1H, J_{gem} = 8.0 Hz, $J_{5'',6''}$ = 4.5 Hz, H-6″b), 3.75-3.69 (m, 3H, H-3′, H-6′a/b), 3.65-3.63 (m, 1H, H-3), 3.62-3.55 (m, 6H, H-2′, H-4, H-4″, OCH_3), 3.31-3.28 (m, 1H, H-2). ^{13}C NMR

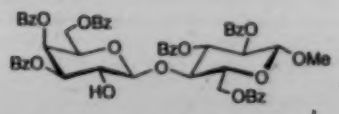
(125 MHz) δ 104.1, 103.9, 101.2, 79.5, 78.2, 76.3, 75.6, 75.3, 73.7, 73.0, 71.8, 71.7, 70.0, 69.8, 69.4, 61.4, 61.2, 60.9, 58.0. ES HRMS Calcd. for $C_{19}H_{34}O_{16}Na$ (M+Na):541.1739. Found 541.1742.



2,3:5,6:3′,4′-Tri-*O*-isopropylidiene-2′-*O*-benzyl-6′-*O*-(1-methoxy-1-methyl) lactose dimethyl acetal (42).

2,3:5,6:3′,4′-Tri-*O*-isopropylidiene-6′-*O*-(1-methoxy-1-methyl) lactose dimethyl acetal **39**[127] (82 mg, 0.14 mmol) was dissolved in DMF (5 ml), and sodium hydride (60 %) (12 mg, 0.5 mmol) was added followed by benzyl bromide (34 ul, 0.29 mmol). The mixture was stirred for 1 h and diluted with DCM before washing with water. The organic layer was dried over sodium sulfate and concentrated to a syrup. Column chromatography of the residue using hexane:EtOAc (1:1) gave **42** (85 mg, 89 %) as a colorless syrup: $[\alpha]_D$ +26.2 (c = 0.1, CH_3CN). ^1H NMR (600 MHz, CD_3CN) δ 7.36-7.25 (m, 5H, Ar), 4.79 (d, 1H, J_{gem} = 12.6, $PhCH_2$), 4.69 (d, 1H, J_{gem} = 12.6, $PhCH_2$), 4.61 (d, 1H, J = 8.4, H-1), 4.39-4.33 (m, 2H, H-a, H-d), 4.23 (dd, 1H, $J_{d,e}$ = 12.0 Hz, $J_{e,f}$ = 6.0 Hz, H-e), 4.16 (dd, 1H, J = 6.4, 1.8 Hz, H-4), 4.11-4.09 (m, 2H, H-3, H-b), 4.06 (dd, 1H, J_{gem} = 9.0 Hz, $J_{e,f}$ = 6.0 Hz, H-fa), 3.94 (dd, 1H, J_{gem} = 8.4 Hz, $J_{e,f}$ = 6.0

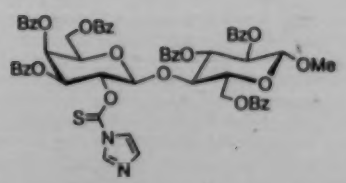
Hz, H-fb), 3.84 (d, 1H, J = 6.0, H-c), 3.81-3.79 (m, 1H, H-5), 3.59-3.51 (m, 2H, H-6a/b), 3.38 (s, 3H, $CHOCH_3$), 3.37 (s, 3H, $COCH_3$), 3.26 (t, 1H, J = 7.5 Hz, H-2), 3.14 (s, 3H, OCH_3), 1.35, 1.34, 1.33, 1.31, 1.29, 1.28 (s x 6, 3H ea, CCH_3), 1.27 (s, 6H, CCH_3). ^{13}C NMR (100 MHz) δ 139.7, 129.1, 129.0, 128.9, 128.8, 110.7, 110.2, 109.2, 106.6, 103.5, 100.9, 81.5, 78.5, 78.4, 77.8, 77.7, 76.9, 76.8, .76.6, 74.2, 72.9, 72.8, 66.6, 60.5, 56.4, 54.5, 28.2, 27.7, 27.6, 27.3, 26.9, 26.4, 24.7, 24.6. ES HRMS Calcd. for $C_{34}H_{54}O_{13}Na$ (M+Na):693.3456. Found 693.3456.



Methyl 3,4,6-tri-*O*-benzoyl-β-*O*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (43).

2,3:5,6:3′,4′-Tri-*O*-isopropylidiene-2′-*O*-benzyl-6′-*O*-(1-methoxy-1-methyl) lactose dimethyl acetal **42** (1.535 g, 2.3 mmol) was dissolved in 60 % aq. acetic acid (20 ml), refluxed for 2h, and concentrated. The residue was dissolved in pyridine (15 ml) and benzoyl chloride (2.5 ml) was added at 0 °C. After stirring overnight at room temperature TLC showed complete conversion. Concentration to dryness gave a syrup to which DCM (3 ml) was added followed by 45 % HBr in acetic acid (2.7 ml). After 2 h the reaction mixture was neutralized by saturated aqueous sodium bicarbonate and washed with brine. Concentration to dryness gave a syrup to which dry methanol (15 ml) was added under argon. After stirring for 24 h the reaction

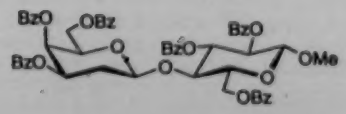
mixture was neutralized with aqueous sodium bicarbonate and wash with brine. Column chromatography of the residue using hexane:EtOAc (3:1) gave **43** (0.483 g, 22 %, 4 steps) as a white solid: $[\alpha]_D$ -7.9 (c = 0.1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 8.08-7.15 (m, 30H, Ar), 5.81 (t, 1H, *J* = 9.6 Hz, H-3), 5.66 (d, 1H, *J* = 3.6 Hz, H-4′), 5.47 (dd, 1H, *J* = 9.6, 7.8 Hz, H-2), 5.23-5.21 (m, 1H, H-3′), 5.03 (d, 1H, J_{gem} = 11.4 Hz, H-6a), 4.68 (d, 1H, *J* = 7.8 Hz, H-1), 4.63-4.60 (m, 2H, H-1′, H-6b), 4.20 (t, 1H, *J* = 9.6 Hz, H-4), 4.08 (dd, 1H, *J* = 10.2, 7.8 Hz, H-2′), 4.03-4.00 (m, 1H, H-5), 3.84-3.81 (m, 1H, H-5′), 3.66 (d, 2H, *J* = 6.6 Hz, H-6′a/b), 3.53 (s, 3H, OCH_3). ^{13}C NMR (125 MHz) δ 166.4, 165.8, 165.6, 165.5, 165.2, 165.1, 133.4, 133.3, 133.2, 133.1, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.1, 128.5, 128.4, 128.3, 128.2, 118.6, 116.9, 103.6, 101.9, 85.7, 76.7, 73.9, 73.2, 71.6, 71.1, 70.0, 67.6, 67.5, 63.1, 60.9, 57.1. Anal. Calcd. for $C_{55}H_{48}O_{17}Si$: C, 67.34; H, 4.93. Found: C, 67.20; H, 5.06. ES HRMS Calcd. for $C_{55}H_{48}O_{17}Na$ (M+Na):1003.2789. Found 1003.2788.



Methyl 2-*O*-(imidazol-1-ylthiocarbonyl)-3,4,6-tri-*O*-benzoyl-β-D-galacto pyranosyl-(1→4)- 2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (44).

Methyl 3,4,6-tri-*O*-benzoyl-β-*O*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside **43** (45 mg, 0.046 mmol) was dissolved in dry toluene (4 ml) at

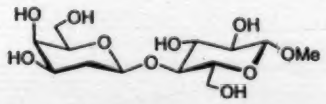
125 °C. Thiocarbonyl diimidazole (17 mg, 0.095 mmol) was added and the reaction stirred under argon for 4 h. Evaporation of the organic solvent gave a dark brown residue that was purified by column chromatography (1:3 EtOAc:Hexane) to give 37 mg (74 %) of compound **44** as a white solid: $[\alpha]_D$ +46.1 (c = 0.2, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 8.59-6.96 (m, 33H, Ar), 6.36 (dd, 1H, *J* = 10.2, 7.8 Hz, H-2′), 5.80 (t, 1H, *J* = 9.6 Hz, H-3), 5.74 (d, 1H, *J* = 3.6 Hz, H-4′), 5.49-5.45 (m, 2H, H-2, H-3′), 4.93 (d, 1H, *J* = 7.8 Hz, H-1′), 4.65 (d, 1H, *J* = 7.8 Hz, H-1), 4.56 (d, 2H, *J* = 3.0, H-6a/b), 4.25 (t, 1H, *J* = 9.6 Hz, H-4), 3.91-3.89 (m, 1H, H-5), 3.86 (t, 1H, *J* = 6.6 Hz, H-5′), 3.74-3.67 (m, 2H, H-6′a/b), 3.51 (s, 3H, OCH_3). ^{13}C NMR (125 MHz) δ 166.0, 165.4, 165.2, 165.1, 165.0, 133.6, 133.5, 133.4, 133.2, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 128.7, 128.6, 128.4, 128.3, 128.2, 102.0, 99.9, 76.7, 75.6, 72.9, 72.8, 71.7, 71.4, 67.7, 62.1, 60.8, 57.2. Anal. Calcd. For $C_{59}H_{50}N_2O_{17}SSi$: C, 64.95; H, 4.62; N, 2.57; S, 2.94. Found: C, 65.02; H, 4.76; N, 2.54; S, 2.66. ES HRMS Calcd. for $C_{59}H_{51}N_2O_{17}S_1Na$ (M+Na):1091.2908. Found 1091.2904.



Methyl 3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-*lyxo*-hexopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (45).

To a solution of **44** (38 mg, 34.8 μmol) in toluene (5 ml) were added tributyltin hydride (14 ul, 52.3 μmol) and 2,2-azobisisobutylronitrile (3 mg, 17.4 μmol). After

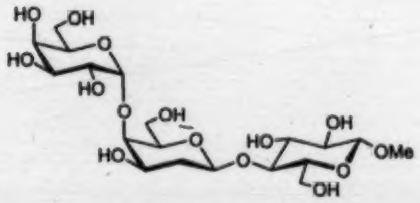
stirring for 3 h, hot acetonitrile (3 ml) and hexane (5 ml) was added. Evaporation of the organic solvents gave a colorless liquid residue that was purified by column chromatography (1:1 EtOAc:hexane) to give 20 mg (61 %) of methyl 2′-deoxy lactoside **45**: $[\alpha]_D$ -10.13 (c = 0.2, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 8.08-7.11 (m, 30H, Ar), 5,78 (t, 1H, *J* = 9.6 Hz, H-3), 5.54 (d, 1H, *J* = 3.0 Hz, H-4′), 5.48 (dd, 1H, *J* = 9.6, 7.8 Hz, H-2), 5.30-5.17 (m, 1H, H-3′), 4.82-4.71 (m, 2H, H-1′, H-6a), 4.69 (d, 1H, *J* = 7.8 Hz, H-1), 4.58 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 4.2 Hz, H-6b), 4.32 (t, 1H, *J* = 9.6 Hz, H-4), 4.00-3.98 (m, 1H, H-5), 3.81-3.72 (m, 3H, H-5′, H-6′a/b), 3.53 (s, 3H, OCH_3), 2.21-2.09 (m, 2H, H-2′a/b). ^{13}C NMR (125 MHz) δ 165.6, 165.3, 165.2, 165.1, 133.4, 133.2, 129.8, 129.6, 129.5, 129.4, 129.3, 129.2, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 102.0, 99.4, 75.4, 75.3, 73.2, 72.9, 71.7, 71.6, 69.0, 65.8, 62.8, 61.6, 57.1, 32.4. Anal. Calcd. for $C_{55}H_{48}O_{16}Si$: C, 68.46; H, 5.01. Found: C, 68.60; H, 5.35. ES HRMS Calcd. for $C_{55}H_{48}O_{16}Na$ (M+Na):987.2840. Found 987.2841.



Methyl 2-deoxy-β-D-*lyxo*-hexopyranosyl-(1→4)-β-D-glucopyranoside (46).

Methyl 3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-*lyxo*-hexopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside **45** (18 mg, 18.7 μmol) was dissolved in methanol (3 ml), 0.5 M sodium methoxide in methanol (37 μl) was added under argon and the

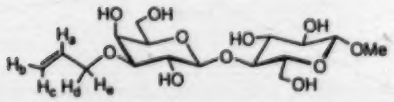
mixture was stirred overnight. The solution was neutralized with Amberlite IR 120 resin and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography in methanol:EtOAc (1:3) to give a white solid (6 mg, quant.): ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.69 (dd, 1H, $J_{1',2'ax}$ = 9.9 Hz, $J_{1',2'eq}$ = 2.4 Hz, H-1'), 4.39 (d, 1H, J = 7.8 Hz, H-1), 3.91-3.86 (m, 2H, H-3', H-6a), 3.81-3.72 (m, 4H, H-4', H-6b, H-6'a/b), 3.67 (t, 1H, J = 9.0 Hz, H-4), 3.63-3.59 (m, 2H, H-3, H-5'), 3.56 (s, 3H, OCH_3), 3.55-3.52 (m, 1H, H-5), 3.30 (t, 1H, J = 8.4 Hz, H-2), 2.09-2.06 (m, 1H, H-2'eq), 1.70 (ddd, 1H, J_{gem} = 12.0 Hz, $J_{2'ax,3'}$ = 12.0 Hz, $J_{1',2'ax}$ = 9.6 Hz, H-2'ax). ^{13}C NMR (125 MHz) δ 103.9, 101.2, 79.1, 76.4, 75.5, 75.2, 73.7, 68.5, 67.5, 62.2, 61.0, 58.1, 34.3. ES HRMS Calcd. for $C_{13}H_{24}O_{10}Na$ (M+Na):363.1261. Found 363.1260.



Methyl α-D-galactopyranosyl-(1→4)-(2-deoxy-β-D-*lyxo*-hexopyranosyl)-(1→4)-β-D-glucopyranoside (20).

The 2'-deoxy lactoside **46** (4.9 mg, 0.017 mmol) was dissolved in water (315 μl). HEPES buffer (100 μl), DTT (25 μl) and alkaline phosphatase (10 μl) were added. To the mixture UDP-Glc (13.2 mg, 0.026 mmol) was added followed by the activated enzyme (50 μl). The reaction was incubated at 37 °C for 2 days then purified with a

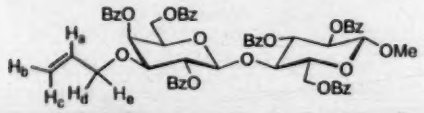
Waters C-18 Sep-Pak solid-phase cartridge (0-50 % MeOH). The product was collected and lyophilized to give a white powder (5 mg, 57 %): $[\alpha]_D$ +44.4 (c = 0.1, H_2O). ^1H NMR (600 MHz, D_2O) δ 4.97 (d, 1H, J = 4.2 Hz, H-1″), 4.77 (dd, 1H, $J_{1',2'ax}$ = 9.6 Hz, $J_{1',2'eq}$ = 2.4 Hz, H-1′), 4.40 (d, 1H, J = 7.8 Hz, H-1), 4.31 (t, 1H, J = 6.6 Hz, H-5′), 4.02 (d, 1H, J = 2.4 Hz, H-4′), 3.95-3.89 (m, 5H, H-3′, H-4″, H-6a, H-6″a/b), 4.85 (dd, 1H, J = 4.2, 1.8 Hz, H-5″), 3.84 (d, 1H, J = 4.2 Hz, H-2″), 3.75-3.65 (m, 5H, H-3″, H-4, H-6b, H-6′a/b), 3.61 (t, 1H, J = 9.0 Hz, H-3), 3.57 (s, 3H, OCH_3), 3.55-3.52 (m, 1H, H-5), 3.29 (dd, 1H, J = 9.6, 8.4 Hz, H-2), 2.11-2.07 (m, 1H, H-2′eq), 1.77 (ddd, 1H, J_{gem} = 12.3 Hz, $J_{2'ax,3'}$ = 12.3 Hz, $J_{1',2'ax}$ = 10.2 Hz, H-2′ax). ^{13}C NMR (125 MHz) δ 103.1, 100.8, 100.2, 78.8, 75.9, 75.8, 74.7, 74.4, 72.9, 70.9, 69.2, 69.0, 68.6, 67.8, 57.2, 34.2. ES HRMS Calcd. for $C_{19}H_{34}O_{15}Na$ (M+Na):525.1789. Found 525.1790.



Methyl 3-*O*-allyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (49).

Methyl lactoside 48[131] (1 g, 2.8 mmol), 3 Å MS (5 g) and dibutyltin oxide (768 mg, 3.1 mmol) were dissolved in acetonitrile (50 ml) and refluxed. After stirring for 24 h, allylbromide (7.12 ml, 84.2 mmol) and tetrabutyl ammoniumbromide (0.5 g, 1.55 mmol) were added and the mixture was refluxed for 48 h. Removal of the volatiles and column chromatography of the residue in methanol:DCM (1:5) gave a

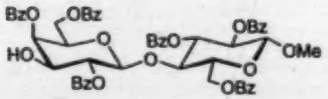
white solid **49** (686 mg, 62 %): $[\alpha]_D$ -3.0 (c = 0.1, CH_3OH). ^1H NMR (600 MHz, CD_3OD) δ 5.98 (dddd, 1H, $J_{Ha,Hc}$ = 17.4 Hz, $J_{Ha,Hb}$ = 10.8 Hz, $J_{Ha,Hd} \approx J_{Ha,He}$ = 6.0 Hz, CH_2=C<u>H</u>CH_2O), 5.32 (dddd, 1H, $J_{Ha,Hc}$ = 17.4 Hz, $J_{Hc,Hb} \approx J_{Hc,Hd} \approx J_{Hc,He}$ = 1.8 Hz, CH_b<u>H</u>$_c$=$CHCH_2O$), 5.16-5.14 (dddd, 1H, C<u>H</u>$_b$$H_c$=$CHCH_2O$), 4.37 (d, 1H, J = 7.8 Hz, H-1′), 4.22 (dddd, 1H, J_{gem} = 12.6 Hz, $J_{Ha,Hd}$ = 6.0 Hz, $J_{Hb,Hd} \approx J_{Hc,Hd}$ = 1.2 Hz, CH_2=CHC<u>H</u>$_d$$H_e$O), 4.19 (d, 1H, J = 7.8 Hz, H-1), 4.12 (dddd, 1H, J_{gem} = 12.8 Hz, $J_{Ha,He}$ = 5.9 Hz, $J_{Hb,He} \approx J_{Hc,He}$ = 1.2 Hz, CH_2=$CHCH_d$<u>H</u>$_e$O), 3.99 (d, 1H, J = 3.0 Hz, H-4′), 3.90 (dd, 1H, J_{gem} = 6.0 Hz, $J_{5,6}$ = 2.4 Hz, H-6a), 3.84 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 4.2 Hz, H-6b), 3.77 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5',6'}$ = 7.8 Hz, H-6′a), 3.70 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5',6'}$ = 4.8 Hz, H-6′b), 3.62 (dd, 1H, J = 7.8 Hz, H-2′), 3.57-3.49 (m, 6H, H-3, H-4, H-5′, C<u>H</u>$_3$O), 3.39 (m, 1H, H-5), 3.32 (d, 1H, J=3.6 Hz, H-3′), 3.22 (dd, 1H, J = 9.0 Hz, $J_{1,2}$ = 7.8 Hz, H-2). ^{13}C NMR (125 MHz) δ 136.4, 117.4, 105.2, 105.0, 91.3, 82.1, 80.7, 76.9, 76.4, 74.7, 71.8, 71.6, 67.0, 62.5, 61.9, 57.3. ES HRMS Calcd. for $C_{16}H_{28}O_{11}Na$ (M+Na):419.1523. Found 419.1527.



Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-allyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (50).

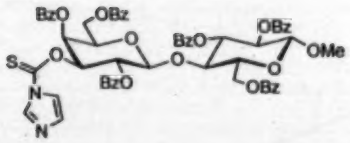
Methyl 3-*O*-allyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **49** (0.686 mg, 1.73 mmol) was dissolved in dry pyridine (10 ml). Benzoyl chloride (1.81 ml, 15.6

mmol) was added and the solution was stirred under argon for 24 h. The reaction mixture was diluted with DCM and washed with 5% HCl, saturated aqueous sodium bicarbonate and brine. Evaporation of the organic solvent gave a light yellow residue that was purified by column chromatography (1:3 EtOAc:hexane) to give 2.4 g (90 %) of **50**: $[\alpha]_D$ +20.6 (c = 0.11, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.18-7.05 (m, 30H, Ar), 5.78 (t, 1H, J = 9.0 Hz, H-3), 5.56-5.50 (m, 2H, H-4′, CH_2=C$\underline{H}_a$$CH_2$O), 5.46-5.42 (m, 2H, H-2, H-2′), 5.07 (dddd, 1H, $J_{Ha,Hc}$ = 17.4 Hz, $J_{Hc,Hb} \approx J_{Hc,Hd} \approx J_{Hc,He}$ = 1.6 Hz,$CH_b\underline{H}_c$=$CHCH_2$O), 4.97 (dddd, 1H, C$\underline{H}_b$$H_c$=$CHCH_2$O), 4.70 (d, 1H, J = 8.4 Hz, H-1′), 4.61-4.57 (m, 2H, H-1, H-6a), 4.50 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 4.2 Hz, H-6b), 4.19 (t, 1H, J = 6.6 Hz, H-4), 4.04 (dddd, 1H, J_{gem} = 12.6 Hz, $J_{Ha,Hd}$ = 6.0 Hz, $J_{Hb,Hd} \approx J_{Hc,Hd}$ = 1.2 Hz, CH_2=CHC$\underline{H}_d$$H_e$O), 3.86-3.82 (m, 2H, H-5, CH_2=CHC$H_d\underline{H}_e$O), 3.78 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5';6'}$ = 6.0 Hz, H-6′a), 3.71-3.69 (m, 1H, H-5′), 3.63 (dd, 1H, $J_{2';3'}$ = 10.2 Hz, $J_{3';4'}$ = 3.6 Hz, H-3′), 3.55 (dd, 1H, J_{gem} = 1.4 Hz, $J_{5';6'}$ = 6.6 Hz, H-6′b), 3.45 (s, 3H, OCH_3). ^{13}C NMR (125 MHz) δ 165.9, 165.7, 165.6, 165.4, 165.2, 164.7, 134.5, 133.8, 133.4, 133.3, 133.2, 133.1, 132.9, 130.6, 130.5, 130.0, 129.8, 129.7, 129.6, 129.5, 129.3, 129.2, 128.8, 128.6, 128.5, 128.4, 128.3, 128.0, 117.6, 114.7, 114.4, 101.9, 101.0, 75.9, 73.0, 72.8, 71.7, 71.5, 71.4, 70.5, 69.4, 66.5, 61.6, 57.1, 57.0. ES HRMS Calcd. for $C_{58}H_{52}O_{17}Na$ (M+Na):1043.3096. Found 1043.3094.



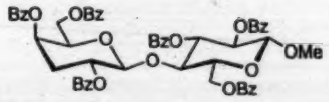
Methyl 2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (51).

The selectively benzylated lactoside **50** (2 g, 2.22 mmol) was dissolved in methanol (30 ml) and the solution was adjusted to pH 3-4 by the addition of acetic acid (11 ml). Palladium (II) chloride (100 mg) was added in several portions. The solution was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:2) to give a yellow solid (1.5 g, 69 %): $[\alpha]_D$ -0.2 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08-7.02 (m, 30H, Ar), 5.75 (t, 1H, J = 9.6 Hz, H-3′), 5.48-5.44 (m, 2H, H-2′, H-4), 5.30 (dd, 1H, J = 10.2, 7.8 Hz, H-2), 4.74 (d, 1H, J = 7.8 Hz, H-1), 4.67 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5',6'}$ = 1.8 Hz, H-6′a), 4.62-4.58 (m, 2H, H-1′, H-6′b), 4.22 (t, 1H, J = 9.6 Hz, H-4′), 3.95-3.91 (m, 1H, H-3), 3.88-3.85 (m, 1H, H-5′), 3.79 (dd, 1H, J = 11.4, 6.0 Hz, H-5), 3.72 (t, 1H, J = 6.0, H-6a), 3.51-3.39 (m, 4H, H-6b, OCH_3). ^{13}C NMR (125 MHz) δ 166.5, 166.0, 165.8, 165.6, 165.4, 165.2, 133.6, 133.5, 133.4, 133.3, 133.1, 133.0, 130.1, 130.0, 129.9, 129.8, 129.6, 129.5, 129.3, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 124.8, 124.5, 102.0, 100.5, 75.8, 73.7, 73.0, 72.7, 71.9, 71.6, 71.5, 70.0, 62.6, 61.4, 57.1. ES HRMS Calcd. for $C_{55}H_{48}O_{17}Na$ (M+Na):1003.2783. Found 1003.2784.



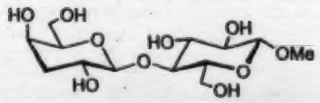
Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-(imidazol-1-ylthiocarbonyl)-β-D-galacto pyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (52).

The selectively deprotected lactoside **51** (1.38 g, 1.41 mmol) was dissolved in dry toluene (20 ml) at 125 °C. Thiocarbonyl diimidazole (502 mg, 2.82 mmol) was added and the reaction was stirred under argon for 4 h. Evaporation of the organic solvent gave a dark brown residue that was purified by column chromatography (1:3 EtOAc:hexane) to give derivative **52** (1.44 g, 94 %) as a white solid: $[\alpha]_D$ +33.4 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11-7.12 (m, 33H, Ar), 5.86 (dd, 1H, J = 3.6, 1.6 Hz, H-4′), 5.82-5.75 (m, 3H, H-2′, H-3, H-3′), 5.46 (dd, 1H, J = 9.6, 7.8 Hz, H-2), 4.91 (d, 1H, J = 7.2 Hz, H-1′), 4.64-4.60 (m, 2H, H-1, H-6a), 4.55 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 4.8 Hz, H-6b), 4.23 (t, 1H, J = 9.6 Hz, H-4), 3.90 (t, 1H, J = 6.6 Hz, H-5′), 3.89-3.84 (m, 1H, H-5), 3.74 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5';6'}$ = 6.6 Hz, H-6′a), 3.66 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5';6'}$ = 7.2 Hz, H-6′b), 3.47 (s, 3H, OCH_3). ^{13}C NMR (125 MHz) δ 166.0, 165.5, 165.3, 165.2, 164.8, 134.1, 133.8, 133.5, 133.4, 133.2, 133.1, 130.0, 129.8, 129.6, 129.5, 129.2, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 101.9, 100.5, 72.9, 72.7, 71.6, 71.1, 69.6, 66.4, 62.5, 62.4, 60.9, 57.1. ES HRMS Calcd. for $C_{59}H_{50}N_2O_{17}SNa$ (M+Na):1113.2722. Found 1113.2724.



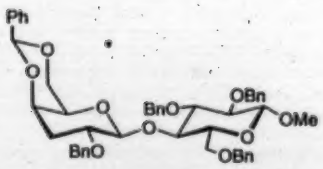
Methyl 2,4,6-tri-*O*-benzoyl-3-deoxy-β-D-*xylo*-hexopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (53).

To a solution of **52** (811 mg, 0.74 mmol) in toluene (10 ml) were added tributyltin hydride (0.3 ml, 1.1 mmol) and 2,2-azobisisobutylronitrile (61 mg, 0.37 mmol). After stirring for 3h, hot acetonitrile and hexane were added. Evaporation of the organic solvent gave a colorless liquid residue that was purified by column chromatography (1:3 EtOAc:hexane) to give 3′-deoxy lactoside **53** (452 mg, 63 %) as a white solid: $[\alpha]_D$ -12.0 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09-7.11 (m, 30H, Ar), 5.74 (t, 1H, $J_{2,3}$ = 9.3 Hz, H-3), 5.45 (dd, 1H, $J_{2,3}$ = 9.3 Hz, $J_{1,2}$ = 7.8 Hz, H-2), 5.21 (br s, 1H, H-4′), 5.19-5.14 (m, 1H, H-2′), 4.75-4.71 (m, 2H, H-1, H-6a), 4.63-4.70 (m, 2H, H-1, H-6b), 4.24 (t, 1H, J = 9.6 Hz, H-4), 3.90-3.87 (m,1H, H-5), 3.84 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5';6'}$ = 6.0 Hz, H-6′a), 3.76 (t, 1H, J = 6.6 Hz, H-5′), 3.55 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5';6'}$ = 6.6 Hz, H-6′b), 3.46 (s, 3H, OCH_3), 2.59-2.56 (m, 1H, H-3′a), 1.77-1.73 (m, 1H, H-3′b). ^{13}C NMR (125 MHz) δ 166.0, 165.7, 165.5, 165.2, 164.9, 133.4, 133.3, 133.2, 133.1, 133.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 102.0, 75.4, 74.7, 73.2, 72.7, 71.7, 68.5, 67.1, 62.7, 61.9, 57.1, 32.9. ES HRMS Calcd. for $C_{55}H_{48}O_{16}Na$ (M+Na):987.2834. Found 987.2837.



Methyl 3-deoxy-β-D-*xylo*-hexopyranosyl-(1→4)-β-D-glucopyranoside (54).

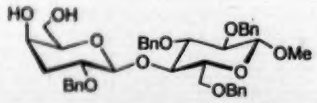
Methyl 2,4,6-tri-*O*-benzoyl-3-deoxy-β-D-*xylo*-hexopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside **53** (437 mg, 0.45 mmol) was dissolved in methanol (10 ml), 0.5 M sodium methoxide in methanol (0.9 ml) was added under argon and the mixture was stirred overnight. The solution was neutralized with Amberlite IR 120 resin and filtered. The solution was removed under reduced pressure and the residue was purified by column chromatography in methanol:EtOAc (1:3) to give a white solid (138 mg, 90 %): $[\alpha]_D$ -23.6 (c = 0.1, H_2O). ^1H NMR (600 MHz, D_2O) δ 4.44 (d, 1H, *J* = 7.8 Hz, H-1′), 4.40 (d, 1H, *J* = 7.8 Hz, H-1), 4.00-3.97 (m, 2H, H-4′, H-5), 3.82-3.57 (11H, H-2′, H-3, H-4, H-5′, H-6a/b, H-6′a/b, OCH_3), 3.57-3.30 (m, 1H, H-2), 2.23-2.19 (m, 1H, H-3′a), 1.76-1.71 (m, 1H, H-3′b). ^{13}C NMR (125 MHz) δ 105.7, 103.9, 79.5, 79.2, 75.6, 75.2, 73.6, 66.5, 66.4, 62.1, 61.0, 58.0, 37.8. ES HRMS Calcd. for $C_{13}H_{24}O_{10}Na$ (M+Na):363.1261. Found 363.1261.



Methyl 2-*O*-benzyl-3-deoxy-4,6-*O*-benzylidene-β-D-*xylo*-hexopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (55).

The 3′-deoxy lactoside **54** (106 mg, 0.31 mmol) was dissolved in CH_3CN (5 ml) and DMF (0.5 ml). Benzaldehyde dimethylacetal (94 µl, 0.62 mmol) and *p*-TsOH (12 mg, 62 µmol) were added and the mixture was stirred for 24 h. The solution was neutralized with triethylamine. The reaction mixture was then concentrated to dryness to give a white solid to which DMF (5 ml) was added followed by benzyl bromide (222 ul, 1.86 mmol) and sodium hydride (75 mg). After stirring for 24 h evaporation of the organic solvent gave a light yellow residue that was purified by column chromatography (1:4 EtOAc:hexane) to give **55** (215 mg, 88 %, 2steps): $[\alpha]_D$ -18.6 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, CD_3Cl) δ 7.52-7.18 (m, 25H, Ar), 5.48 (s, 1H, PhCH), 5.21 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.91 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.82 (d, 1H, J_{gem} = 10.2 Hz, $PhCH_2$), 4.77 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.69-4.67 (m, 1H, $PhCH_2$), 4.60-4.56 (m, 3H, H-1′, $PhCH_2$), 4.45 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.35 (d, 1H, J = 7.8 Hz, H-1), 4.21 (dd, 1H, J_{gem} = 13.2 Hz, $J_{5',6'}$ = 1.2 Hz, H-6′a), 4.02 (t, 1H, J = 9.6 Hz, H-3), 3.97 (br s, 1H, H-4′), 3.92-3.83 (m, 3H, H-5, H-6a, H-6′b), 3.71-3.66 (m, 2H, H-2′, H-4), 3.59 (s, 3H, OCH_3), 3.48-3.45 (m, 2H, H-2, H-6b), 3.15 (s, 1H, H-5′), 2.42-2.38 (m, 1H, H-3′a), 1.61-1.56 (m, 1H, H-3′b). ^{13}C NMR (125 MHz) δ 139.0, 138.7, 138.5, 138.2, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2,

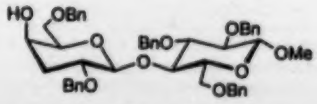
128.1, 128.0, 127.6, 127.5, 127.4, 127.3, 127.2, 126.4, 104.7, 101.3, 83.1, 81.9, 77.4, 75.7, 75.2, 74.9, 73.7, 73.2, 73.0, 72.7, 69.1, 69.0, 68.5, 57.0, 34.9. ES HRMS Calcd. for $C_{48}H_{52}O_{10}Na$ (M+Na):811.3452. Found 811.3453.



Methyl 2-*O*-benzyl-3-deoxy-β-D-*xylo*-hexopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (56).

Protected lactoside **55** (82 mg, 0.1 mmol) was stirred with 80 % acetic acid (2 ml) at 65 °C. After stirring for 4 h the mixture was diluted with DCM and extracted with saturated aqueous sodium bicarbonate. The organic phase was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:3) give to diol **56** (56mg, 77%) as a colorless syrup: $[\alpha]_D$ +7.9 (c = 0.2, $CHCl_3$). ^{1}H NMR (600 MHz, CD_3Cl) δ 7.39-7.23 (m, 20H, Ar), 4.49 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.91-4.84 (m, 2H, $PhCH_2$), 4.75 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.67 (d, 1H, J_{gem} = 11.5 Hz, $PhCH_2$), 4.61-4.57 (m, 2H, $PhCH_2$), 4.49-4.45 (m, 2H, H-1′, $PhCH_2$), 4.33 (d, 1H, J = 8.0 Hz, H-1), 3.96 (t, 1H, J = 9.0 Hz, H-4), 3.86-3.80 (m, 3H, H-4′, H-6a/b), 3.63-3.57 (m, 6H, H-2′, H-3, H-6'a, OCH_3), 3.50-3.42 (m, 3H, H-2, H-5, H-6'b), 3.17-3.15 (m, 1H, H-5′), 2.28-2.24 (m, 1H, H-3′a), 1.45-1.39 (m, 1H, H-3'b). ^{13}C NMR (125 MHz) δ 139.2, 138.6, 138.3, 128.3, 128.2, 128.1, 128.0, 127.5, 127.4, 127.3, 127.0, 104.7, 104.6, 82.9, 81.7, 76.5, 76.1, 74.8, 73.5, 73.1, 72.5, 68.3,

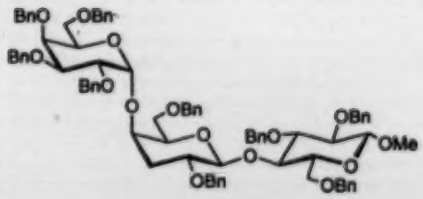
63.5, 57.0, 36.6. Anal. Calcd. for $C_{41}H_{48}O_{10}Si$: C, 70.27; H, 6.90. Found: C, 69.95; H, 7.09. ES HRMS Calcd. for $C_{41}H_{48}O_{10}Na$ (M+Na):723.3140. Found 723.3139.



Methyl 2,6-di-*O*-benzyl-3-deoxy-β-D-*xylo*-hexopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (57).

Diol **56** (47 mg, 67 μmol) was dissolved in DCM (3 ml) and 5 % aqueous sodium hydroxide was added (0.2 ml). Benzyl bromide (17 μl, 0.12 mmol) and tetrabutylammonium hydrogensulfate (5 mg, 13.4 μl) were added and stired for 36 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:3) to give **57** (36 mg, 68 %) as a colorless syrup: $[\alpha]_D$ +3.1 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, CD_3Cl) δ 7.35-7.20 (m, 25H, Ar), 5.00 (d, 1H, J_{gem} = 11.4 Hz, $PhCH_2$), 4.87 (d, 1H, J_{gem} = 11.4 Hz, $PhCH_2$), 4.81 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.61-4.51 (m, 4H, H-1′, $PhCH_2$), 4.46 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.39 (s, 2H, $PhCH_2$), 4.32 (d, 1H, J = 7.8 Hz, H-1), 3.99-3.92 (m, 2H, H-4, H-4′), 3.83-3.82 (m, 2H, H-6a/b), 3.63-3.55 (m, 5H, H-2′, H-3, OCH_3), 3.50-3.36 (m, 5H, H-2, H-5, H-5′, H-6′a/b), 2.32-2.29 (m, 1H, H-3′a), 1.47-1.42 (m, 1H, H-3′b). ^{13}C NMR (125 MHz) δ 139.3, 138.6, 138.4, 137.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.1, 104.7, 104.6, 83.0, 81.8, 76.6, 75.7, 75.1, 75.0, 74.8, 73.8, 73.5, 73.1, 72.4, 69.4, 68.6,

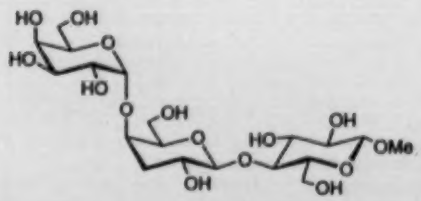
66.9, 57.0, 36.2. ES HRMS Calcd. for $C_{48}H_{54}O_{10}Na$ (M+Na):813.3609. Found 813.3608.



Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-(2,6-di-*O*-benzyl-3-deoxy-β-D-*xylo*-hexopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (58).

A mixture of glycosyl acceptor **57** (36 mg, 45.5 μmol), thioglycoside donor **34** (65 mg, 0.1 mmol), and crushed 4 Å MS (500 mg) were stirred in dry DCM (5 ml) at room temperature under argon for 1 h. The mixture was treated with *N*-iodosuccinimide (15 mg, 0.67 mmol) and trifluoromethanesulfonic acid (1 μl, 11 μmol). After 30 min the mixture was diluted with DCM, filtered through Celite, and this solution was washed sequentially with saturated sodium thiosulfate, saturated sodium bicarbonate, water, and brine. Organic solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:3) give to **58** (26 mg, 43 %): ^{1}H NMR (600 MHz, CD_3Cl) δ 7.43-7.08 (m, 45H, Ar), 5.07-5.04 (m, 2H, H-1″, $PhCH_2$), 4.91 (d, 1H, J_{gem} = 11.4 Hz, $PhCH_2$), 4.86 (d, 1H, J_{gem} = 11.4 Hz, $PhCH_2$), 4.77-4.70 (m, 4H, $PhCH_2$), 4.63 (d, 1H, J_{gem} = 11.4 Hz, $PhCH_2$), 4.57-4.53 (m, 6H, H-1′, $PhCH_2$), 4.46-4.42 (m, 2H, $PhCH_2$), 4.35 (d, 1H,

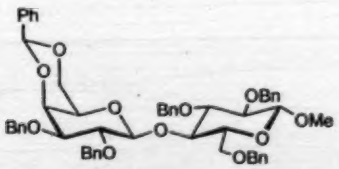
J_{gem} = 12.0 Hz, $PhCH_2$), 4.32 (d, 1H, J = 7.2 Hz, H-1), 4.26-4.21 (m, 2H, $PhCH_2$), 4.04 (dd, 1H, J = 10.2, 3.6 Hz, H-2″), 3.98-3.94 (m, 3H, H-4, H-4″, H-6a), 3.89-3.79 (m, 5H, H-3″, H-4′, H-6′a, H-6″a.b), 3.61 (t, 1H, J = 9.0 Hz, H-3), 3.58-3.54 (m, 4H, H-2, OCH_3), 3.51-3.42 (m, 5H, H-5, H-5′, H-5″, H-6b, H-6′b), 3.39 (dd, 1H, J = 11.4, 7.8 Hz, H-2), 2.62-2.58 (m, 1H, H-3′a), 1.48-1.42 (m, 1H, H-3′b). ES HRMS Calcd. for $C_{82}H_{88}O_{15}Na$ (M+Na):1335.6015. Found 1335.6018.



Methyl α-D-galactopyranosyl-(1→4)-(3-deoxy-β-D-*xylo* hexopyranosyl)-(1→4)-β-D-glucopyranoside (21).

Protected trisaccharide **58** (26mg, 19.8 μmol) was dissolved in methanol (5 ml). Palladium hydroxide (12 mg) was added under argon and the mixture was stirred for 2 days. The reaction was filtered and purified with a Waters C-18 Sep-Pak solid-phase cartridge (0-50 % MeOH). The product was collected and lyophilized to give a white powder (9 mg, 90 %): $[\alpha]_D$ +38.0 (c = 0.1, H_2O). ^1H NMR (500 MHz, CD_3Cl) δ 5.01 (d, 1H, J = 4.4 Hz, H-1″), 4.50 (d, 1H, J = 8.0 Hz, H-1′), 4.41 (d, 1H, J = 8.0 Hz, H-1), 4.01- 3.97 (m, 4H, H-4′, H-4″, H-5, H-6″a), 3.89-3.85 (m, 2H, H-3″, H-6′a), 3.83-3.79 (m, 4H, H-2″, H-5″, H-6′b, H-6″b), 3.77-3.72 (m, 3H, H-2′, H-5′, H-6a), 3.68-3.63 (m, 2H, H-3, H-6b), 3.62-3.57 (m, 4H, H-4, OCH_3), 3.35-3.29 (m, 1H, H-2),

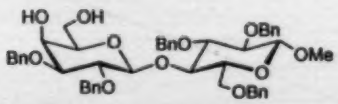
2.43-2.39 (m, 1H, H-3'a), 1.75 (m, 1H, H-3'b). ^{13}C NMR (125 MHz) δ 105.6, 103.9, 101.5, 79.4, 79.3, 75.8, 75.6, 75.3, 73.7, 72.6, 70.1, 70.0, 69.4, 66.9, 62.1, 61.6, 60.9, 58.0, 36.7. ES HRMS Calcd. for $C_{19}H_{34}O_{15}Na$ (M+Na):525.1789. Found 525.1789.



Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (59).

Methyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **48** (400 mg, 1.12 mmol) was dissolved in CH_3CN (7 ml) and DMF (0.4 ml). Benzaldehyde dimethylacetal (0.34 ml, 2.24 mmol) and *p*-TsOH (20 mg, 0.1 mmol) were added and the mixture was stirred for 30 min. The solution was neutralized with triethylamine. The reaction mixture was then concentrated to dryness to give a white solid to which DMF (5 ml) was added followed by benzyl bromide (0.35 ml, 2.94 mmol) and sodium hydride (119 mg). After stirring for 2 h evaporation of the organic solvent gave a light yellow residue that was purified by column chromatography (1:3 EtOAc:hexane) to give 249 mg (70 %, 2 steps) of **59**: $[\alpha]_D$ +16.9 (c=0.3, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53-7.16 (m, 30H, Ar), 5.43 (s, 1H, PhCH), 5.15 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.86-4.80 (m, 2H, $PhCH_2$), 4.76-4.69 (m, 5H, $PhCH_2$), 4.53 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.44 (d, 1H, J = 7.5 Hz, H-1'), 4.32-4.27 (m, 2H, H-1, $PhCH_2$), 4.18 (dd, 1H,

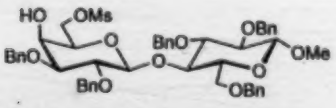
J_{gem} = 12.5 Hz, $J_{5',6'}$ = 1.5 Hz, H-6'a), 4.00 (d, 1H, J = 3.5 Hz, H-4'), 3.96 (t, 1H, J = 9.5 Hz, H-4), 3.88-3.80 (m, 2H, H-6'b, H-6a), 3.75-3.69 (m, 2H, H-2', H-6), 3.60 (t, 1H, J = 9.0 Hz, H-3), 3.54 (s, 3H, OCH_3), 3.41-3.32 (m, 3H, H-2, H-3', H-5), 2.90 (s, 1H, H-5'). ^{13}C NMR (125 MHz) δ 139.0, 138.9, 138.7, 138.6, 138.4, 138.1, 128.8, 128.6, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 126.5, 104.7, 102.8, 101.3, 83.0, 81.9, 79.7, 78.8, 77.6, 75.7, 75.3, 75.1, 74.9, 73.7, 73.0, 71.6, 68.9, 68.3, 66.3, 57.0. ES HRMS Calcd. for $C_{55}H_{58}O_{11}Na$ (M+Na):917.3871. Found 917.3873.



Methyl 2,3,-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (60).

The 4,6-benzylidene lactoside **59** (300 mg, 0.33 mmol) was stirred with 80 % acetic acid (5 ml) at 65 °C. After stirring for 5 h the mixture was diluted with DCM and extracted with saturated aqueous $NaHCO_3$. The solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:2) to give diol **60** (270mg, quant.) as a colorless syrup: $[\alpha]_D$ +23.38 (c = 0.1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 7.19-7.21 (m, 25H, Ar), 4.98 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.88 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.79-4.66 (m, 6H, $PhCH_2$), 4.57 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.39-4.36 (m, 2H, H-1', $PhCH_2$), 4.30 (d, 1H, J =

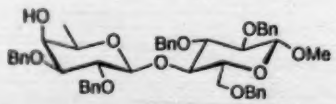
7.7 Hz, H-1), 3.91 (t, 1H, J = 9.0 Hz, H-4), 3.87 (d, 1H, J = 2.4 Hz, H-4′), 3.81 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5,6}$ = 4.2 Hz, H-6a), 3.73 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 1.8 Hz, H-6b), 3.63-3.53 (m, 7H, H-2′, H-3, H-6′a/b, OCH_3), 3.42-3.37 (m, 2H, H-2, H-5), 3.33 (dd, 1H, J = 9.6, 3.6 Hz, H-3′), 3.14-3.12 (m, 1H, H-5′). ^{13}C NMR (125 MHz) δ 138.9, 138.6, 138.2, 137.8, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 104.7, 102.5, 82.7, 81.7, 80.9, 79.2, 76.8, 75.5, 75.2, 75.0, 74.8, 73.9, 73.1, 72.1, 68.1, 67.2, 62.3, 57.0. ES HRMS Calcd. For $C_{48}H_{54}O_{11}Na$ (M+Na):829.3558. Found 829.3556.



Methyl 2,3-di-*O*-benzyl-6-*O*-mesyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (61).

Diol **60** (48 mg, 59.5 μmol) was stirred with pyridine (2 ml) at 0 °C. MsCl was added and the solution was stirred for 1 h. The mixture was diluted with ethyl acetate (10 ml) and extracted with 1 M sulfuric acid solution and with saturated aqueous sodium bicarbonate. The solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:1) to give derivative **61** (39 mg, 74%): ^1H NMR (600 MHz, $CDCl_3$) δ 7.42-7.21 (m, 25H, Ar), 4.89-4.86 (m, 2H, $PhCH_2$), 4.78-4.62 (m, 7H, $PhCH_2$), 4.39-4.37 (m, 2H, H-1′, $PhCH_2$), 4.29 (d, 1H, J = 8.2 Hz, H-1), 4.20-4.14 (m, 2H, H-6′a/b), 3.93 (t, 1H, J = 9.0 Hz, H-4), 3.86 (dd, 1H,

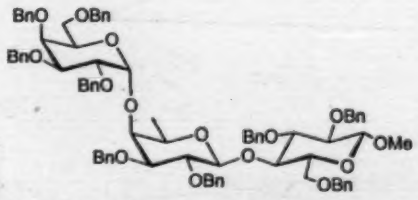
J = 3.6, 1.2 Hz, H-4′), 3.83 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 3.6 Hz, H-6a), 3.70 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 1.8 Hz, H-6b), 3.57-3.54 (m, 4H, H-2′, OCH_3), 3.50 (t, 1H, J = 9.0 Hz, H-3), 3.37-3.31 (m, 4H, H-2, H-3′, H-5, H-5′), 2.70 (s, 3H, SO_2CH_3). ES HRMS Calcd. for $C_{49}H_{56}O_{13}SNa$ (M+Na):907.3333. Found 907.3332.



Methyl 2,3-di-*O*-benzyl-β-D-fucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-gluco-pyranoside (63).

The selectively protected disaccharide **61** (30 mg, 33.9 μmol) was dissolved in THF (2 ml), lithium aluminium hydride (2 mg, 50 μmol) was added under argon and the mixture was stirred for 3 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:2) to give 6′-deoxy lactoside **63** (26 mg, 96%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45-7.21 (m, 25H, Ar), 5.02 (d, 1H, J_{gem} = 10.8, $PhCH_2$), 4.86 (d, 1H, J_{gem} = 11.4, $PhCH_2$), 4.78-4.77 (m, 3H, $PhCH_2$), 4.72-4.65 (m, 3H, $PhCH_2$), 4.58 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.42-4.39 (m, 2H, H-1′, $PhCH_2$), 4.30 (d, 1H, J = 7.8 Hz, H-1), 4.00 (t, 1H, J = 9.6 Hz, H-4), 3.84 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 4.2 Hz, H-6a), 3.75 (d, 1H, J_{gem} = 10.8 Hz, H-6b), 3.66 (d, 1H, J = 2.4 Hz, H-4′), 3.60-3.56 (m, 4H, H-3, OCH_3), 3.51 (app t, 1H, J = 9.0 Hz, H-2′), 3.44-3.34 (m, 3H, H-2, H-3′, H-5), 3.24 (q, 1H, J = 6.6 Hz, H-5′),

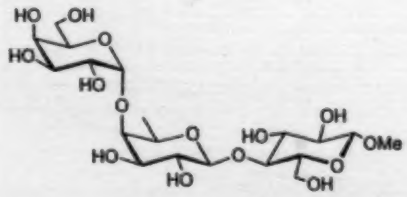
1.21 (d, 3H, J = 6.6 Hz, H-6′). ES HRMS Calcd. for $C_{48}H_{54}O_{10}Na$ (M+Na):813.3609. Found 813.3606.



Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-(2,3-di-*O*-benzyl-β-D-fucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (64).

A mixture of glycosyl acceptor **64** (31 mg, 31.6 μmol), thioglycoside donor **34** (31 mg, 47.9 μmol), and crushed 4 Å MS (300 mg) was stirred in dry DCM (3 ml) at room temperature under argon for 1 h. The mixture was treated with *N*-iodosuccinimide (11 mg, 48.9 μmol) and trifluoromethanesulfonic acid (2 μl, 2.3 μmol). After 30 minues the mixture was diluted with DCM, filtered through Celite, and the solution was washed sequentially with saturated sodium thiosulfate, saturated sodium bicarbonate, water, and brine. The organic solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:4) to give trisaccharide **64** (38 mg, 92 %) as a colorless syrup: $[\alpha]_D$ +36.2 (c = 0.24, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48-7.09 (m, 40H, Ar), 5.12 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.97 (d, 1H, J_{gem} = 3.5 Hz, H-1″), 4.88 (d, 1H, J_{gem} = 11.5 Hz, $PhCH_2$), 4.83-4.80 (m, 2H, $PhCH_2$), 4.76-4.66 (m, 6H, $PhCH_2$), 4.56-4.47 (m, 5H, $PhCH_2$), 4.43 (d, 1H, J = 8.0 Hz, H-1′), 4.35 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$),

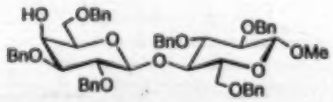
4.32-4.30 (m, 1H, H-5″), 4.28 (d, 1H, J = 8.0 Hz, H-1), 4.17-4.12 (m, 2H, $PhCH_2$), 4.06 (dd, 1H, J = 10.0, 3.5 Hz, H-2″), 3.98-3.94 (m, 3H, H-3″, H-4, H-4″), 3.84 (dd, 1H, J_{gem} = 11.0 Hz, $J_{5,6}$ = 4.5 Hz, H-6a), 3.74 (d, 1H, J = 9.5 Hz, H-6b), 3.62 (s, 1H, H-4′), 3.60-3.53 (m, 5H, H-2′, H-3, OCH_3), 3.47 (t, 1H, J = 8.5 Hz, H-6″a), 3.40-3.35 (m, 2H, H-2, H-5), 3.26 (dd, 1H, J = 10.5, 3.0 Hz, H-3′), 3.22-3.18 (m, 2H, H-5′, H-6″b), 1.37 (d, 3H, J = 6.5 Hz, H-6′). Anal. Calcd. for $C_{82}H_{88}O_{15}$: C, 74.98; H, 6.75. Found: C, 74.59; H, 6.81. ES Calcd.:1335.6 found 1335.6.



Methyl α-D-galactopyranosyl-(1→4)-(β-D-fucopyranosyl)-(1→4)-β-D-gluco-pyranoside (22).

Protected trisaccharide **64** (38 mg, 28.9 μmol) was dissolved in methanol (5 ml), Palladium hydroxide (12 mg) under argon was added and the mixture was stirred for 2 days. The reaction was filtered and purified with a Waters C-18 Sep-Pak solid-phase cartridge (0-50 % MeOH). The product was collected and lyophilized to give a white powder (15 mg, 100 %): $[\alpha]_D$ +55.7 (c = 0.1, H_2O). ^1H NMR (500 MHz, $CDCl_3$) δ 5.04 (d, 1H, J = 4.8 Hz, H-1″), 4.46 (d, 1H, J = 8.0 Hz, H-1′), 4.43-4.39 (m, 2H, H-1, H-5″), 4.04 (d, 1H, J = 3.0 Hz, H-4″), 4.00 (d, 1H, J = 12.5 Hz, H-5), 3.93 (dd, 1H, J = 10.5, 3.0 Hz, H-3″), 3.89-3.80 (m, 4H, H-2″, H-4, H-4′, H-5′), 3.74-3.67

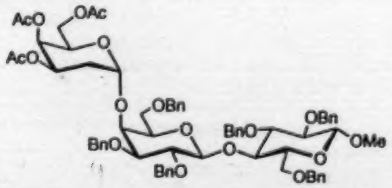
(m, 3H, H-3′, H-6″a/b), 3.64-3.57 (m, 7H, H-2′, H-3, OCH_3), 3.53 (dd, 1H, J = 10.0, 7.5 Hz, H-2′), 3.30 (t, 1H, J = 8.5 Hz, H-2), 1.36 (d, 3H, J = 6.5 Hz, H-6′). ^{13}C NMR (125 MHz) δ 104.1, 103.8, 101.4, 80.0, 79.9, 75.6, 75.3, 73.7, 73.1, 72.2, 71.4, 71.3, 70.0, 69.8, 69.7, 61.3, 60.9, 58.0, 16.3. ES HRMS Calcd. for $C_{19}H_{34}O_{15}Na$ (M+Na):525.1789. Found 525.1793.



Methyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (65).

The disaccharide benzylidene acetal **59** (103 mg, 0.12 mmol) was dissolved in THF. Sodium cyanoborohydride (100 mg) was added and the mixture was stirred with 3 Å MS (100 mg). Saturated HCl (5 ml) in ether was added dropwise over 5 min. The mixture was extracted with saturated aqueous sodium bicarbonate. The solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:2) to give **65** (89mg, 86%): $[\alpha]_D$ +20.33 (c = 0.15, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 7.39-7.17 (m, 30 H, Ar), 4.98 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.86 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.79-4.70 (m, 5H, $PhCH_2$), 4.66 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.56 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.46-4.38 (m, 4H, H-1′, $PhCH_2$), 4.29 (d, 1H, J = 7.2 Hz, H-1), 4.02 (d, 1H, J = 2.4 Hz, H-4′), 3.97 (t, 1H, J = 9.0 Hz, H-3), 3.81 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 4.2 Hz, H-

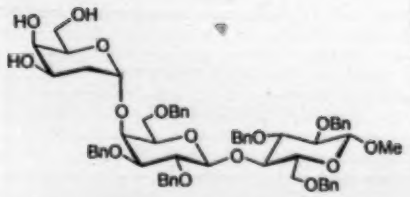
6a), 3.73 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5,6}$ = 1.8 Hz, H-6b), 3.66 (dd, 1H, J_{gem} = 10.2 Hz, $J_{5',6'}$ = 7.2 Hz, H-6'a), 3.60-3.55 (m, 5H, H-2', H-4, OCH_3), 3.48 (dd, 1H, J_{gem} = 9.6 Hz, $J_{5',6'}$ = 5.4 Hz, H-6'b), 3.40-3.35 (m, 3H, H-2, H-3', H-5), 3.31 (t, 1H, J = 6.0 Hz, H-5'). ^{13}C NMR (125 MHz) δ 139.1, 138.7, 138.6, 138.3, 138.2, 137.9, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 104.6, 102.5, 82.8, 81.8, 81.1, 79.4, 76.5, 75.3, 75.2, 75.1, 74.8, 73.5, 73.1, 72.7, 72.0, 68.4, 68.2, 66.1, 57.0. Anal. Calcd. for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.54; H, 6.80. ES HRMS Calcd. for $C_{55}H_{60}O_{11}Na$ (M+Na):919.4027. Found 919.4023.



Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*lyxo*-hexopyranosyl-(1→4)-(2,3,6-*O*-tribenzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (73).

Glycosyl acceptor **65** (67 mg, 74.7 μmol), thioglycoside donor **72**[136] (150 mg, 0.4 mmol), and crushed 4 Å MS (700 mg) were stirred in dry DCM (7 ml) at 0 °C under argon for 1 h. The mixture was treated with *N*-iodosuccinimide (53 mg, 0.24 mmol) and trifluoromethanesulfonic acid (3 μl, 15 μmol). After 24 h the mixture was diluted with DCM, filtered through Celite, and the solution was washed sequentially with saturated sodium thiosulfate, saturated sodium bicarbonate, water, and brine. The solvent was removed under reduced pressure and the residue was purified by

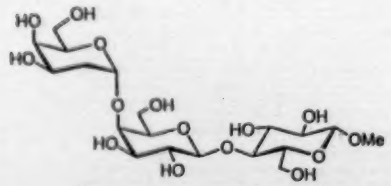
column chromatography in EtOAc:hexane (1:5) to give trisaccharide **73** (53 mg, 61 %): $[\alpha]_D$ +59.1 (c = 0.2, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 7.40-7.18 (m, 30 H, Ar), 5.30 (br s, 1H, H-4″), 5.27-5.23 (ddd, 1H, H-3″), 5.05 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 5.01 (d, 1H, J = 3.0 Hz, H-1″), 4.91 (d, 1H, J_{gem} = 11.4 Hz, $PhCH_2$), 4.85-4.82 (m, 2H, $PhCH_2$), 4.78 (d, 1H, J_{gem} = 12.6 Hz, $PhCH_2$), 4.72-4.69 (m, 2H, $PhCH_2$), 4.64 (d, 1H, J_{gem} = 12.6, $PhCH_2$), 4.56-4.53 (m, 2H, H-5″, $PhCH_2$), 4.40-4.28 (m, 5H, H-1′, H-1, $PhCH_2$), 4.06 (d, 1H, J=3.0 Hz, H-4′), 3.98-3.91 (m, 2H, H-4, H-6″a), 3.81-3.79 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 4.2 Hz, H-6a), 3.70 (dd, 1H, J_{gem} = 10.8 Hz, H-6b), 3.63-3.60 (dd, 1H, J = 9.6, 7.8 Hz, H-2′), 3.53-3.51 (m, 5H, H-3, H-6″b, OCH_3), 3.39-3.31 (m, 3H, H-3′, H-5, H-6′a), 3.26-3.20 (m, 3H, H-2, H-5′, H-6′b), 2.09 (s, 3H, $COCH_3$), 1.95 (ddd, 1H, J_{gem} = 12.6 Hz, J = 3.6 Hz, H-2″a), 1.91 (s, 3H, $COCH_3$), 1.86 (s, 3H, $COCH_3$) 1.63 (ddd, 1H, J_{gem} = 12.6 Hz, J = 4.8 Hz, H-2″b). ^{13}C NMR (125 MHz) δ 170.3, 170.1, 169.9, 139.5, 138.7, 138.6, 138.3, 138.2, 137.4, 128.5, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.4, 127.3, 127.1, 126.8, 104.6, 102.7, 98.8, 82.9, 81.6, 80.1, 79.3, 76.7, 75.1, 75.0, 74.8, 73.5, 73.3, 73.0, 72.3, 72.1, 68.1, 67.2, 66.4, 66.3, 66.0, 61.1, 57.0, 30.2, 20.8, 20.7, 20.6. ES HRMS Calcd. for $C_{67}H_{76}O_{18}Na$ (M+Na):1191.4923. Found 1191.4928.



Methyl 2-deoxy-α-D-*lyxo*-hexopyranosyl-(1→4)-(2,3,6-*O*-tri-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (74).

Trisaccharide **73** (88 mg, 75.3 μmol) was dissolved in methanol (5 ml), 0.5 M sodium methoxide in methanol (226 μl) was added under argon and the mixture was stirred overnight. The solution was neutralized with Amberlite IR 120 resin and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (3:2) to give **74** (58 mg, 73 %): $[\alpha]_D$ +35.0 (c = 0.1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 7.38-7.19 (m, 30H, Ar), 5.05 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.94 (d, 1H, J = 3.0 Hz, H-1″), 4.85-4.69-1, (m, 6H, $PhCH_2$), 4.62 (d, 1H, J_{gem} = 12.6 Hz, $PhCH_2$), 4.56 (d, 1H, J_{gem} = 12.6 Hz, $PhCH_2$), 4.42-4.36 (m, 3H, H-1′, $PhCH_2$), 4.31-4.28 (m, 2H, H-1, $PhCH_2$), 4.02 (dd, 1H, J = 3.0 Hz, H-4′), 3.99 (t, 1H, J = 3.0 Hz, H-5″), 3.95 (dd, 1H, $J_{3,4} \approx J_{4,5}$ = 9.0 Hz, H-4), 3.90-3.86 (m, 1H, H-3″), 3.84 (br s, 1H, H-4″), 3.81 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 4.2 Hz, H-6a), 3.72 (d, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 1.8 Hz, H-6b), 3.57-3.50 (m, 5H, H-2′, H-3, OCH_3), 3.46-3.44 (m, 2H, H-6″a/b), 3.40-3.24 (m, 6H, H-2, H-3′, H-5′, H-5, H-6′a/b), 1.74 (ddd, 1H, J_{gem} = 12.6 Hz, J = 3.6 Hz, H-2″a), 1.62 (ddd, 1H, J_{gem} = 12.6 Hz, J = 4.8 Hz, H-2″b). ^{13}C NMR (125 MHz) δ 139.4, 138.6, 138.4, 138.3, 138.2, 137.5, 128.5, 128.3,

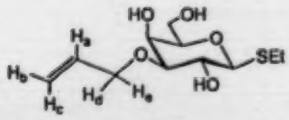
128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 127.1, 104.6, 102.6, 99.4, 87.5, 82.9, 81.6, 80.7, 80.6, 79.2, 75.1, 75.0, 74.8, 73.5, 73.1, 72.4, 70.3, 69.1, 68.2, 67.2, 65.3, 64.0, 63.2, 57.0, 33.1. ES HRMS Calcd. For $C_{61}H_{70}O_{15}Na$ (M+Na):1065.4606. Found 1065.4609.



Methyl 2-deoxy-α-D-*lyxo*-hexopyranosyl-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (23)

Protected trisaccharied **74** (59 mg, 56.6 μmol) was dissolved in 5 ml methanol. Palladium hydroxide was added and the suspension was stirred for 24 h. The reaction was filtered and purified with a Waters C-18 Sep-Pak solid-phase cartridge (0-50 % MeOH). The product was collected and lyophilized to give a white powder (29 mg, 100 %): $[\alpha]_D$ +27.2 (c = 0.1, H_2O). ^1H NMR (500 MHz, $CDCl_3$) δ 5.04-5.02 (m, 1H, H-1″), 4.48 (d, 1H, *J* = 7.5 Hz, H-1′), 4.41 (d, 1H, *J* = 8.0 Hz, H-1), 4.23 (t, 1H, *J* = 6.5 Hz, H-5″), 4.10 (td, 1H, *J* = 8.5, 3.0 Hz, H-3″), 4.03-3.98 (m, 2H, H-4′, H-5), 3.91 (d, 1H, *J* = 3.0 Hz, H-4″), 3.82 (dd, 1H, J_{gem} = 13.0 Hz, *J* = 5.0 Hz, H-6a), 3.77-3.75 (m, 3H, H-6b, H-6′a, H-6″a), 3.73-3.70 (m, 3H, H-3, H-3′, H-6″b), 3.68-3.63 (m, 2H, H-5′, H-6′b), 3.62-3.56 (m, 4H, H-4, OCH_3), 3.53 (dd, 1H, *J* = 12.0, 8.0 Hz, H-2′), 3.32-3.29 (m, 1H, H-2), 1.96-1.93 (m, 2H, H-2″a/b). ^{13}C NMR (125 MHz) δ 103.9,

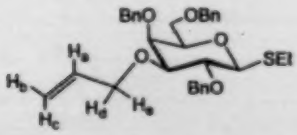
103.9, 100.3, 79.1, 77.7, 76.2, 75.6, 75.3, 73.6, 72.9, 72.1, 72.0, 68.3, 65.5, 61.9, 61.4, 60.8, 58.1, 32.3. ES HRMS Calcd. for $C_{19}H_{34}O_{15}Na$ (M+Na):525.1789. Found 525.1789.



Ethyl 3-*O*-allyl-1-thio-β-D-galactopyranoside (81)

Ethyl 1-thio-β-D-galactopyranoside **80**[135] (5.45 g, 24.3 mmol) and dibutyltin oxide (6.21 g, 25.0 mmol) was dissolved in benzene (100 ml) and refluxed. After stirring for 17 h, allylbromide (3.11ml, 36.8 mmol) and tetrabutyl ammoniumbromide (9.11g, 28.3 mmol) were added and the reaction mixture was heated (63 °C) for 6 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography in ethyl acetate to give **81** as a white solid (3.85 g, 60 %): $[\alpha]_D$ -13.8 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.96 (dddd, 1H, $J_{Ha,Hc}$ = 17.4 Hz, $J_{Ha,Hb}$ = 10.2 Hz, $J_{Ha,Hd} \approx J_{Ha,He}$ = 6.0 Hz, CH_2=C<u>H</u>CH_2O), 5.33 (dddd, 1H, $J_{Ha,Hc}$ = 17.4 Hz, $J_{Hc,Hb} \approx J_{Hc,Hd} \approx J_{Hc,He}$ = 1.8 Hz, CH_b<u>H_c</u>=$CHCH_2O$), 5.24-5.22 (dddd, 1H, C<u>H_b</u>H_c=$CHCH_2O$), 4.32 (d, 1H, *J*=9.6 Hz, H-1), 4.24-4.22 (m, 2H, CH_2=CH<u>CH_2</u>O), 4.08 (d, 1H, $J_{3,4}$ = 3.0, H-4), 3.96 (dd, 1H, J_{gem} = 11.4 Hz, J_{gem} = 6.6 Hz, H-6a), 3.82-3.76 (m, 2H, H-2, H-6b), 3.55 (ddd, 1H, *J* = 6.5, 4.8, 1.2 Hz, H-5), 3.39 (dd, 1H, *J* = 8.4, $J_{3,4}$ = 3.0 Hz, H-3), 2.80-2.70 (m, 2H, S<u>CH_2</u>CH_3), 1.315 (t, 3H, *J* = 6.0 Hz, SCH_2<u>CH_3</u>). ^{13}C NMR (125 MHz) δ 134.3, 118.0, 86.2, 81.1, 78.3, 71.0, 69.1, 67.3,

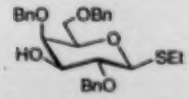
62.7, 24.1, 15.3. Anal. Calcd. for $C_{11}H_{20}O_5S$: C, 49.98; H, 7.63; S, 12.13. Found: C, 50.00; H, 7.62; S, 12.18. ES HRMS Calcd. for $C_{11}H_{20}O_5SNa$ (M+Na):287.0923. Found 287.0925.



Ethyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (82).

Allyl galactoside **81** (1.6 g, 6.1 mmol) was dissolved in DMF (10 ml) under argon. To the ice-cold solution, sodium hydride (1.1 g, 27.3 mmol) was added portionwise. After stirring for 30 min at room temperature, benzyl bromide (3.3 ml, 27.3 mmol) was added dropwise. The reaction mixture was quenched with water, then diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate, and brine, and dried over sodium sulfate. After removal of the solvent, the crude material was chromatographed on silica (1:9 Ethyl acetate:hexane) to give **82** (2.377 g, 73 %): $[\alpha]_D$ -1.78 (c = 0.18, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43-7.25 (m, 15H, Ar), 5.95 (dddd, 1H, $J_{Ha,Hc}$ = 16.8 Hz, $J_{Ha,Hb}$ = 10.2 Hz, $J_{Ha,Hd} \approx J_{Ha,He}$ = 5.4 Hz, CH_2=$\underline{CH}CH_2O$), 5.33 (dddd, 1H, $J_{Ha,Hc}$ = 17.4 Hz, $J_{Hc,Hb} \approx J_{Hc,Hd} \approx J_{Hc,He}$ = 1.8 Hz, $CH_b\underline{H_c}$=$CHCH_2O$), 5.20-5.18 (dddd, 1H, $C\underline{H_b}H_c$=$CHCH_2O$), 4.94 (d, 1H, J_{gem} = 11.4 Hz, $PhCH_2$), 4.85 (d, 1H, J_{gem} = 10.2, $PhCH_2$), 4.79 (d, 1H, J_{gem} = 10.2 Hz, $PhCH_2$), 4.61 (d, 1H, J_{gem} = 12.0, $PhCH_2$), 4.47-4.40 (m, 3H, H-1, $PhCH_2$), 4.20-4.18 (m, 2H, CH_2=$CH\underline{CH_2}O$), 3.93 (d, 1H, J = 2.4 Hz, H-4), 3.77 (t, 1H, J = 9.6 Hz, H-2), 3.61-3.54 (m, 3H, H-5, H-6a/b),

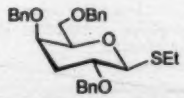
3.46 (dd, 1H, J = 9.0, 2.4 Hz, H-3), 2.79-2.67 (m, 2H, $S\underline{CH_2}CH_3$), 1.29 (t, 3H, J = 7.5 Hz, $SCH_2\underline{CH_3}$). ^{13}C NMR (125 MHz) δ 138.8, 138.3, 137.8, 134.8, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.4, 116.7, 85.2, 83.8, 78.3, 77.2, 75.7, 74.3, 73.5, 73.4, 71.5, 68.8, 24.7, 15.0. ES HRMS Calcd. for $C_{32}H_{38}O_5SNa$ (M+Na):557.2332. Found 557.2332.



Ethyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (83).

The protected allyl ether **82** (1.5 g, 2.8 mmol), tris(triphenylphosphine)rhodium (I) chloride (417 mg, 0.45 mmol) and 1,4-diazabicyclo[2,2,2]octane (168 mg, 1.5 mmol) were dissolved in 7:3:1 EtOH:toluene:water (77 ml) and refluxed for 1 h. Then this solution was cooled down and filtered through Celite. The solvent were removed under reduced pressure. A solution of the residue in 9:1 1 M HCl:acetone (30 ml) was heated for 24 h at 60 °C and neutralized with saturated aqueous sodium bicarbonate. After removal of the solvent, the crude material was chromatographed on silica (1:11 ethyl acetate:toluene) to give **83** (1.086 g, 78 %): $[\alpha]_D$ +1.85 (c = 0.1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 7.42-7.25 (m, 15H,Ar), 4.94 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.73-4.65 (m, 3H, $PhCH_2$), 4.51 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.46 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.41 (d, 1H, J = 9.6 Hz, H-1), 3.91 (d, 1H, J = 3.0 Hz, H-4), 3.68-3.64 (m, 4H, H-3, H-5, H-6a/b), 3.55 (t, 1H, J = 9.6 Hz, H-2), 2.79-2.70 (m, 2H,

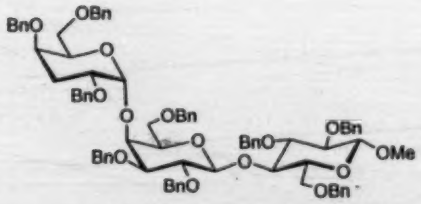
$S\underline{CH_2}CH_3$), 1.31 (t, 3H, J=7.8 Hz, $SCH_2\underline{CH_3}$). ^{13}C NMR (125 MHz) δ 141.6, 128.4, 128.0, 112.9, 106.2, 94.5, 92.0, 87.0, 76.1, 75.8, 73.5, 60.4, 51.9, 44.8, 13.7. ES HRMS Calcd. for $C_{29}H_{34}O_5SNa$ (M+Na):517.2019. Found 517.2018.



Ethyl 2,3,6-tri-*O*-benzyl-3-deoxy-1-thio-β-D-*xylo*-hexopyranoside (84).

A solution of **83** (1.05 g, 2.1 mmol), *N,N*-dimethyl-4-aminopyridine (3.9 g, 31.9 mmol) and phenyl thionochloroformate (0.9 ml, 6.4 mmol) in DCM (20 ml) was stirred for 24 h at 0 °C under argon. The solution was washed with saturated aqueous sodium bicarbonate and concentrated. To a solution of the residue in toluene (20 ml) were added tributyltin hydride (1.7 ml, 6.4 mmol) and 2,2-azobisisobutylronitrile (175 mg, 1.1 mmol). After stirring for 3h, hot acetonitrile (10 ml) and hexane (15 ml) was added. Evaporation of the organic solvents gave a colorless syrup that was purified by column chromatography (1:9 EtOAc:hexane) to give 3-deoxy galactose **84** (328 mg, 33 %, 2 steps): $[\alpha]_D$ -65.1 (c = 0.1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 7.39-7.23 (m, 15H,Ar), 4.71 (d, 1H, J_{gem} = 11.5 Hz, $PhCH_2$), 4.56-4.46 (m, 5H, H-1, H-4, H-5, H-6a/b), 4.36 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 3.71-3.60 (m, 5H, H-2, $PhCH_2$), 2.94-2.68 (m, 2H, $S\underline{CH_2}CH_3$), 2.43 (ddd, 1H, J_{gem} = 13.6 Hz, $J_{2,3} \approx J_{3,4}$ = 4.0 Hz, H-3eq), 1.46 (ddd, 1H, J_{gem} = 13.6 Hz, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 2.5 Hz, H-3ax), 1.31 (t, 3H, J = 7.3 Hz, $SCH_2\underline{CH_3}$). ^{13}C NMR (125 MHz) δ 138.3, 138.1, 128.4, 128.3,

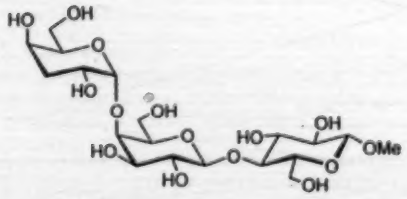
128.2, 128.1, 127.8, 127.7, 127.6, 86.8, 79.0, 73.5, 72.8, 72.5, 71.1, 69.3, 33.9, 31.9, 24.5, 15.0. Anal. Calcd. for $C_{29}H_{34}O_4S$: C, 72.77; H, 7.16; S, 6.70. Found: C, 72.55; H, 7.13; S, 6.76. ES HRMS Calcd. for $C_{29}H_{34}O_4SNa$ (M+Na):501.2070. Found 501.2069.



Methyl 2,4,6-tri-*O*-benzyl-3-deoxy-α-D-*xylo*-hexopyranosyl-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (78).

Glycosyl acceptor **65** (70 mg, 78.1 μmol), glycosyl donor **84** (81 mg, 0.17 mmol), and crushed 4 Å MS (500 mg) were stirred in dry DCM (5 ml) at 0 °C under argon for 1 h. The mixture was treated with *N*-iodosuccinimide (26 mg, 0.11 mmol) and trifluoromethanesulfonic acid (1 μl, 0.2 μmol). After 24 h the mixture was diluted with DCM, filtered through Celite, and the organic solution was washed sequentially with saturated sodium thiosulfate, saturated sodium bicarbonate, water, and brine. The organic solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:4) to give trisaccharide **78** (50 mg, 49 %): $[\alpha]_D$ +12.9 (c = 0.1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 7.39-

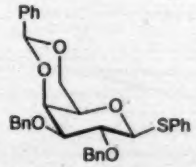
7.11 (m, 45H,Ar), 5.06 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 5.03 (d, 1H, J = 3.0 Hz, H-1″), 4.85-4.80 (m, 3H, $PhCH_2$), 4.74-4.65 (m, 3H, $PhCH_2$), 4.52-4.41 (m, 6H, H-1′, H-5, $PhCH_2$), 4.38-4.26 (m, 6H, H-1, $PhCH_2$), 4.18-4.09 (m, 4H, H-4′, H-5′, $PhCH_2$), 4.93 (t, 1H, J = 9.0 Hz, H-4), 3.87 (ddd, 1H, $J_{2'',3''ax}$ = 12.0 Hz, $J_{1'',2''} \approx J_{2'',3''eq}$ = 3.6 Hz, H-2″), 3.81 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5'',6''}$ = 4.2 Hz, H-6″a), 3.73-3.71 (m, 2H, H-4″, H-6″b), 3.65 (dd, 1H, J = 9.6, 7.2 Hz, H-2′), 3.57-3.52 (m, 5H, H-3, H-6a, OCH_3), 3.46 (dd, 1H, J_{gem} = 9.0 Hz, $J_{5',6'}$ = 4.8 Hz, H-6′a), 3.38-3.30 (m, 4H, H-2, H-3′, H-5″, H-6′b), 3.16 (dd, 1H, J_{gem} = 8.4 Hz, $J_{5,6}$ = 4.8 Hz, H-6b), 2.04-2.01 (m, 1H, H-3″eq), 1.88 (ddd, 1H, $J_{gem} \approx J_{2'',3''ax}$ = 12.8 Hz, $J_{3'',4''}$ = 2.4 Hz, H-3″ax). ^{13}C NMR (125 MHz) δ 139.3, 138.7, 138.6, 138.5, 138.4, 138.3, 136.4, 133.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.0, 104.6, 102.8, 99.5, 82.7, 81.7, 79.4, 75.1, 74.8, 74.4, 73.1, 73.0, 71.9, 71.3, 70.7, 69.2, 68.3, 68.2, 67.6, 38.3. ES HRMS Calcd. for $C_{82}H_{88}O_{15}Na$ (M+Na):1335.6015. Found 1335.6014.



Methyl 3-deoxy-α-D-*xylo*-hexopyranosyl-(1→4)-(β-D-galactopyranosyl)- (1→4)-β-D-glucopyranoside (24).

The protected trisaccharide **78** (40 mg, 30.5 μmol) was dissolved in methanol (5 ml). Palladium hydroxide (10 ml) was added and the suspension was stirred for 24 h. The

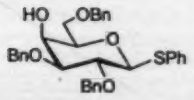
reaction mixture was filtered and purified with a Waters C-18 Sep-Pak solid-phase cartridge (0-50 % MeOH). The filtrate was collected and lyophilized to give a white powder (14 mg, 93 %): $[\alpha]_D$ +33.8 (c = 0.1, H_2O). ^1H NMR (600 MHz, $CDCl_3$) δ 4.89 (d, 1H, J = 3.6 Hz, H-1″), 4.52 (d, 1H, J = 7.8 Hz, H-1′), 4.41 (d, 1H, J = 7.8 Hz, H-1), 4.25 (t, 1h, J = 6.6 Hz, H-5″), 4.10-4.05 (m, 3H, H-2″, H-4″, H-5′), 4.01 (dd, 1H, J = 12.6, 1.2 Hz, H-3′), 3.92 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5,6}$ = 7.8 Hz, H-6a), 3.85-3.79 (m, 3H, H-4′, H-5, H-6b), 3.75 (dd, 1H, J_{gem} = 10.2 Hz, $J_{5';6'}$ = 3.0 Hz, H-6′a), 3.70-3.56 (m, 9H, H-2′, H-3, H-4, H-6′b, H-6″a/b, OCH3), 3.32-3.29 (m, 1H, H-2), 2.04-1.95 (m, 2H, H-3″a/b). ^{13}C NMR (125 MHz) δ 104.1, 103.9, 100.6, 79.5, 78.4, 76.3, 75.6, 75.3, 73.7, 73.2, 71.9, 71.6, 66.6, 64.6, 61.8, 61.4, 60.9, 58.0, 33.5. ES HRMS Calcd. for $C_{19}H_{34}O_{15}Na$ (M+Na):525.1789. Found 525.1789.



Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (85).

Phenyl galactoside **69**[134] (588 mg, 2.16 mmol) was dissolved in CH_3CN (8 ml)-DMF (2 ml). Benzaldehydedimethylacetal (0.81 ml, 5.4 mmol) and *p*-TsOH (74 mg, 0.4 mmol) were added and the mixture was stirred for 2 h. The solution was neutralized with triethylamine. The reaction mixture was then concentrated to dryness to give a white solid to which DMF (7 ml) was added followed by benzyl bromide (0.77 ml,

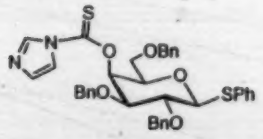
19.4 mmol) and sodium hydride (260 mg). After stirring for 2 h evaporation of the organic solvent gave a light yellow residue that was purified by column chromatography (1:3 EtOAc:hexane) to give **85** (945 mg, 81 %, 2 steps) as a white solid: $[\alpha]_D$ -15.4 (c = 0.16, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72-7.18 (m, 20H, Ar), 5.50 (s, 1H, PhCH), 4.75-4.69 (m, 4H, $PhCH_2$), 4.63 (d, 1H, J = 9.6 Hz, H-1), 4.38 (dd, 1H, J_{gem} = 12.3 Hz, $J_{5,6}$ = 1.8 Hz, H-6a), 4.17 (d, 1H, J = 3.0 Hz, H-4), 4.00 (dd, 1H, J_{gem} = 12.3 Hz, $J_{5,6}$ = 1.2 Hz, H-6b), 3.91 (t, 1H, J = 9.6 Hz, H-2), 3.64 (dd, 1H, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 3.42 (s, 1H, H-5). ^{13}C NMR (125 MHz) δ 138.5, 138.1, 137.9, 132.8, 132.7, 129.0, 128.8, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.4, 126.6, 101.3, 86.5, 81.4, 75.4, 73.7, 71.8, 69.8, 69.4. Anal. Calcd. for $C_{33}H_{32}O_5S$: C, 73.31; H, 5.97; S, 5.93. Found: C, 72.91; H, 6.17; S, 5.66. ES HRMS Calcd. for $C_{33}H_{32}O_5SNa$ (M+Na):563.1862. Found 563.1865.



Phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (86).

Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside **85** (443 mg, 0.82 mmol) was dissolved in THF. Sodium cyanoborohydride (443 mg) was added and the mixture was stirred with 3 Å MS (443 mg). Saturated HCl in ether (2 ml) was added dropwise over 5 min. The mixture was extracted with saturated aqueous sodium bicarbonate. The solution was removed under reduced pressure and the

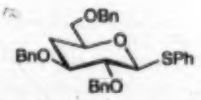
residue was purified by column chromatography in EtOAc:hexane (1:3) to give **86** (324mg, 73%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59-7.23 (m, 15H, Ar), 4.84 (d, 1H, J_{gem} = 10.0 Hz, $PhCH_2$), 4.77-4.64 (m, 4H, H-1, $PhCH_2$), 4.57 (s, 2H, $PhCH_2$), 4.11 (br s, 1H, H-4), 3.83-3.74 (m, 3H, H-2, H-6a/b), 3.62-3.57 (m, 2H, H-3, H-5). ES HRMS Calcd. for $C_{33}H_{34}O_5SNa$ (M+Na):565.2019. Found 565.2019.



Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-(imidazole-1-ylthiocarbonyl)-1-thio-β-D-galactopyranoside (87).

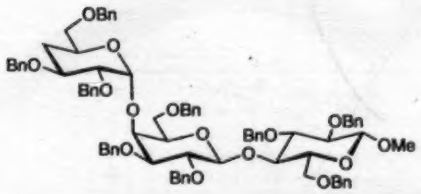
Phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside **86** (320 mg, 0.6 mmol) was dissolved in dry toluene (20 ml) at 125 °C. Thiocarbonyl diimidazole (316 mg, 1.8 mmol) was added and the reaction mixture was stirred under argon for 24 h. Evaporation of the organic solvent gave a dark brown residue that was purified by column chromatography (1:3 EtOAc:hexane) to give **87** (324 mg, 84 %): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (s, 1H, NCH), 7.62-7.60 (m, 2H, Ar), 7.41-7.23 (m, 19H, Ar), 7.02 (s, 1H, NCH), 6.40 (d, 1H, J = 2.4 Hz, H-4), 4.85-4.80 (m, 2H, $PhCH_2$), 4.74 (d, 1H, J_{gem} = 10.2 Hz, $PhCH_2$), 4.68 (d, 1H, J = 9.6 Hz, H-1), 4.54 (d, 1H, J_{gem} = 10.8 Hz, $PhCH_2$), 4.47 (s, 2H, $PhCH_2$), 3.91 (t, 1H, J = 6.6 Hz, H-5), 3.81 (dd, 1H, J = 9.6, 3.6 Hz, H-3), 3.67 (dd, 1H, J_{gem} = 9.6 Hz, $J_{5,6}$ = 6.0 Hz, H-6a), 3.57 (t, 1H, J =

9.6 Hz, H-2), 3.51 (dd, 1H, J_{gem} = 9.6 Hz, $J_{5,6}$ = 7.2 Hz, H-6b). ES HRMS Calcd. for $C_{37}H_{37}N_2O_5SNa$ (M+Na):653.2138. Found 653.2135.



Phenyl 2,3,6-tri-*O*-benzyl-4-deoxy-1-thio-β-D-galactopyranoside (88).

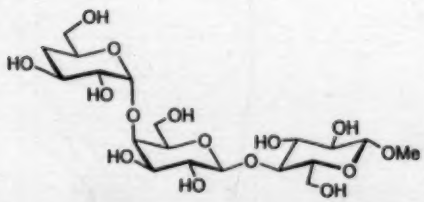
To a solution of **87** (324 mg, 0.5 mmol) in toluene (12 ml) were added tributyltin hydride (0.2 ml, 0.75 mmol) and 2,2-azobisisobutylronitrile (41 mg, 0.25 mmol). After stirring for 35 min, hot acetonitrile (5 ml) and hexane (5 ml) were added. Evaporation of the organic solvent gave a colorless liquid that was purified by column chromatography (1:3 EtOAc:hexane) to give 185 mg (71 %) of 4-deoxy derivative **88**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57-7.20 (m, 20H, Ar), 4.86-4.81 (m, 2H, $PhCH_2$), 4.69-4.63 (m, 2H, H-1, $PhCH_2$), 4.58-4.53 (m, 2H, $PhCH_2$), 3.69-3.63 (m, 3H, H-3, H-5, H-6a), 3.53 (dd, 1H, J_{gem} = 12.9 Hz, $J_{5,6}$ = 7.5 Hz, H-6b), 3.39 (dd, 1H, J = 9.6, 9.0 Hz, H-2), 2.21 (ddd, 1H, J = 12.6, 4.8 Hz, H-4a), 1.53-1.50 (m, 1H, H-4b). ES HRMS Calcd. for $C_{33}H_{34}O_4SNa$ (M+Na):549.2070. Found 549.2071.



Methyl 2,3,6-tri-*O*-benzyl-4-deoxy-α-D-*xylo*-hexopyranosyl-(1→4)-(2,3,6-*O*-tri-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (89).

A mixture of glycosyl acceptor **65** (407 mg, 0.45 mmol), thioglycoside donor **88** (159 mg, 0.3 mmol), and crushed 4 Å MS (1 g) were stirred in dry DCM (15 ml) at room temperature under argon for 1 h. The mixture was treated with *N*-iodosuccinimide (119 mg, 0.52 mmol) and trifluoromethanesulfonic acid (6 µl, 6.8 µmol). After 10 min the mixture was diluted with DCM, filtered through Celite, and the solution was washed sequentially with saturated sodium thiosulfate, saturated sodium bicarbonate, water, and brine. The organic solution was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:3) to give 4″-deoxy trisaccharide **89** (138 mg, 35 %): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43-7.13 (m, 45H, Ar), 5.10-5.08 (m, 2H, H-1″, $PhCH_2$), 4.86-4.83 (m, 2H, $PhCH_2$), 4.80-4.65 (m, 6H, $PhCH_2$), 4.57-4.55 (m, 2H, $PhCH_2$), 4.49-4.45 (m, 3H, H-1′, $PhCH_2$), 4.21-4.36 (m, 3H, $PhCH_2$), 4.34-4.26 (m, 3H, H-1, H-5″, $PhCH_2$), 4.23-4.17 (m, 2H, $PhCH_2$), 4.06 (d, 1H, J = 1.8 Hz, H-4′), 4.00-3.94 (m, 2H, H-3, H-3″), 3.85-3.72 (m, 2H, H-6a/b), 3.64 (dd, 1H, J = 9.6, 7.8 Hz, H-2′), 3.61 (t, 1H, J = 6.0 Hz, H-4), 3.56 (s, 3H, OCH_3), 3.52-3.48 (m, 3H, H-2″, H-5′, H-6′a), 3.38-3.37 (m, 2H, H-2, H-5), 3.34-3.27

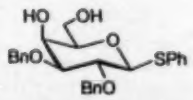
9m, 2H, H-3′, H-6′b), 3.13 (d, 1H, J = 4.2 Hz, H-6″a/b), 2.07-2.04 (ddd, 1H, H-4″a), 1.60 (ddd, 1H, $J_{Gem} \approx J_{3'',4''} \approx J_{4'',5''}$ = 12.0 Hz, H-4″b). ES HRMS Calcd. for $C_{82}H_{88}O_{15}Na$ (M+Na):1335.6020. Found 1335.6021.



Methyl 4-deoxy-α-D-*xylo*-hexopyranosyl-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (25).

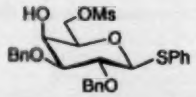
The protected trisaccharide **89** (63mg, 48.0 μmol) was dissolved in methanol (5 ml), Palladium hydroxide was added under argon and the mixture was stirred for 22 h. The solution was filtered and purified with a Waters C-18 Sep-Pak solid-phase cartridge (0-50 % MeOH). The product was collected and lyophilized to give a white powder (11 mg, 46 %): $[\alpha]_D$ +37.1 (c = 0.1, H_2O). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.95 (d, 1H, J = 3.5 Hz, H-1″), 4.50 (d, 1H, J = 8.0 Hz, H-1′), 4.40 (d, 1H, J = 8.5 Hz, H-1), 4.39-4.34 (m, 1H, H-5″), 4.02-3.91 (m, 4H, H-3″, H-4′, H-6a, H-6′a), 3.85-3.81 (m, 2H, H-6b, H-6′b), 3.78 (dd, 1H, J = 7.5, 4.5 Hz, H-5′), 3.74 (dd, 1H, J = 11.0, 3.5 Hz, H-3′), 3.67-3.55 (m, 9H, H-2′, H-3, H-4, H-5, H-6″a/b, OCH_3), 3.48 (dd, 1H, J = 9.5, 3.5 Hz, H-2″), 3.32-3.28 (m, 1H, H-2), 2.20-1.98 (ddd, 1H, H-4″a), 1.49 (ddd, 1H, $J_{Gem} \approx J_{3'',4''} \approx J_{4'',5''}$ = 12.0 Hz, H-4″b). ^{13}C NMR (125 MHz) δ 104.1, 103.9, 101.6, 79.5,

78.1, 76.3, 75.6, 75.3, 74.4, 73.7, 73.0, 71.8, 69.9, 67.7, 61.2, 60.9, 58.0, 34.9. ES HRMS Calcd. for $C_{19}H_{34}O_{15}Na$ (M+Na):525.1789. Found 525.1793.



Phenyl 2,3-di-*O*-benzyl-1-thio-β-D-galactopyranoside (90).

Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside **85** (200 mg, 0.37 mmol) was stirred with 80 % acetic acid (4 ml) at 65 °C. After stirring for 24 h the mixture was diluted with ethyl acetate (15 ml) and extracted with saturated aqueous sodium bicarbonate. The solvent was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (2:3) to give diol **90** (113mg, 68%): $[\alpha]_D$ -0.5 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57-7.24 (m, 15H, Ar), 4.84 (d, 1H, J_{gem} = 10.2 Hz, $PhCH_2$), 4.77-4.66 (m, 4H, H-1, $PhCH_2$), 4.06 (d, 1H, J = 2.4 Hz, H-4), 3.97 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6}$ = 7.2 Hz, H-6a), 3.81-3.60 (m, 2H, H-2, H-6b), 3.58 (dd, 1H, J = 8.4, 5.4 Hz, H-3), 3.49 (ddd, 1H, H-5). Anal. Calcd. For $C_{26}H_{28}O_5S$: C, 69.00; H, 6.24; S, 7.09. Found: C, 68.64; H, 6.33; S, 6.87. ES HRMS Calcd. for $C_{26}H_{28}O_5SNa$ (M+Na):475.1549. Found 475.1549.

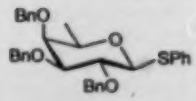


Phenyl 2,3-di-*O*-benzyl-6-*O*-mesyl-1-thio-β-D-galactopyranoside (91).

Diol **90** (372 mg, 0.82 mmol) was stirred with pyridine (5 ml) at 0 °C. Methansulfonyl chloride (76 ul, 9.8 umol) was added and the solution was stirred for 24 h. The mixture was diluted with ethyl acetate (10 ml) and extracted with 1M sulfuric acid and with saturated aqueous sodium bicarbonate. After removal of the organic solvent the residue was purified by column chromatography using EtOAc:hexane (1:1) to give **91** (392mg, 90%): $[\alpha]_D$ -1.45 (c = 0.3, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 7.54-7.25 (m, 15H, Ar), 4.84 (d, 1H, J_{gem} = 10.0 Hz, $PhCH_2$), 4.75-4.67 (m, 4H, H-1, $PhCH_2$), 4.51 (dd, 1H, J_{gem} = 11.0 Hz, $J_{5,6}$ = 7.5 Hz, H-6a), 4.38 (dd, 1H, J_{gem} = 11.0 Hz, $J_{5,6}$ = 4.5 Hz, H-6b), 4.01 (br s, 1H, H-4), 3.75-3.71 (m, 2H, H-2, H-5), 3.62 (dd, 1H, J = 9.0, 3.5 Hz, H-3), 2.95 (s, 3H, SO_2CH_3). ^{13}C NMR (125 MHz) δ 137.9, 137.2, 133.5, 131.5, 129.0, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.5, 87.5, 81.9, 75.8, 75.5, 75.4, 72.6, 68.8, 66.3, 37.4. ES HRMS Calcd. For $C_{27}H_{30}O_7S_2Na$ (M+Na):553.1325. Found 553.1324.

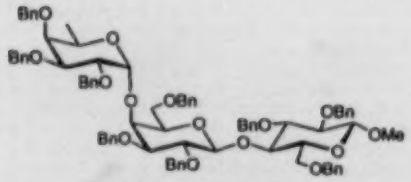
Phenyl 2,3-di-*O*-benzyl-1-thio-β-D-fucopyranoside (94).

Phenyl 2,3-di-*O*-benzyl-6-*O*-methanesulphonyl-1-thio-β-D-galactopyranoside **91** (382 mg, 0.72 mmol) was dissolved in THF (10 ml). Lithium aluminium hydride (43 mg, 1.1 mmol) was added under argon and the mixture was stirred for 4 h. The solution was removed under reduced pressure and the residue was purified by column chromatography in EtOAc:hexane (1:3) to give the 1-thio phenyl fucopyranoside **94** (279 mg, 89%): $[\alpha]_D$ +1.71 (c=0.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 7.59-7.56 (m, 2H, Ar), 7.42-7.23 (m, 13H, Ar), 4.84 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.76-4.68 (m, 3H, $PhCH_2$), 4.60 (d, 1H, J = 10.0 Hz, H-1), 4.83 (d, 1H, J = 2.0 Hz, H-4), 3.70 (dd, 1H, J = 9.3 Hz, H-2), 3.59-3.55 (m, 2H, H-3, H-5), 1.38 (d, 3H, J = 6.5 Hz, H-6). ^{13}C NMR (125 MHz) δ 138.2, 137.6, 133.9, 131.9, 128.8, 128.5, 128.3, 128.2, 128.0, 127.8, 127.7, 127.3, 87.5, 82.9, 76.7, 75.7, 74.2, 69.4, 16.7. ES HRMS Calcd. for $C_{26}H_{28}O_4SNa$ (M+Na):459.1600. Found 459.1603.



Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-fucopyranoside (95).

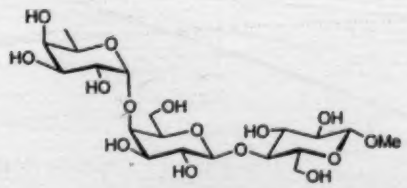
Phenyl 2,3-di-*O*-benzyl-1-thio-β-D-fucopyranoside **94** (313 mg, 0.72 mmol) was dissolved in DMF (10 ml) under argon. To the ice-cold solution NaH (43 mg, 1.1 mmol) was added portionwise. After stirring for 30 min at room temperature, benzyl bromide (128 ul, 1.1 mmol) was added dropwise. The reaction mixture was quenched with water, then diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate, brine, and dried over sodium sulfate. After removal of the solvent, the crude material was chromatographed on silica (1:4 EtOAc:hexane) to give **95** (332 mg, 88 %): $[\alpha]_D$ +4.0 (c = 0.14, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60-7.58 (m, 2H, Ar), 7.40-7.27 (m, 15H, Ar), 7.22-7.19 (m, 3H, Ar), 5.02 (d, 1H, J_{gem} = 12 Hz, $PhCH_2$), 4.81-4.73 (m, 4H, $PhCH_2$), 4.68 (d, 1H, J_{gem} = 11.4 Hz, $PhCH_2$), 4.61 (d, 1H, J = 9.6 Hz, H-1), 3.93 (dd, 1H, J = 9.6 Hz, H-2), 3.65 (d, 1H, J = 2.4 Hz, H-4), 3.61 (dd, 1H, J = 9.6, 2.7 Hz, H-3), 3.54 (q, 1H, J = 6.6 Hz, H-5), 1.28 (d, 3H, J = 6.6 Hz, CH_3). ^{13}C NMR (125 MHz) δ 138.7, 138.4, 138.3, 131.5, 128.7, 128.4, 128.3, 128.2, 127.9, 127.6, 127.5, 127.4, 126.9, 87.5, 84.5, 75.5, 74.6, 72.8, 63.3, 59.1, 33.6. Anal. Cald. For $C_{33}H_{34}O_4S$: C, 75.25; H, 6.51; S, 6.09. Found: C, 75.35; H, 6.58; S, 6.25. ES HRMS Calcd. for $C_{33}H_{34}O_4SNa$ (M+Na):549.2070. Found 549.2071.



Methyl 2,3,4-tri-*O*-benzyl-α-D-fucopyranosyl-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (96).

A mixture of glycosyl acceptor **65** (119 mg, 0.13 mmol), phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-fucopyranoside **95** (70 mg, 0.13 mmol), and crushed 4 Å MS (500 mg) in dry DCM (5 ml) was stirred at room temperature under argon for 1 h. The mixture was treated with *N*-iodosuccinimide (45 mg, 0.2 mmol) and trifluoromethanesulfonic acid (2.4 µl, 2.7 µmol). After 30 minutes the mixture was diluted with DCM, filtered through Celite, and the solution was washed sequentially with saturated sodium thiosulfate, saturated sodium bicarbonate, water, and brine. The organic layer was concentrated to dryness and the residue was purified by column chromatography using EtOAc:hexane (1:4) to give trisaccharide **96** (153 mg, 87 %): $[\alpha]_D$ +46.9 (c = 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40-7.11 (m, 45H, Ar), 5.06 (d, 1H, J = 10.8 Hz, $PhCH_2$), 5.03 (d, 1H, J = 3.5 Hz, H-1″), 4.90 (d, 1H, J_{gem} = 10.0 Hz, $PhCH_2$), 4.84-4.72 (m, 7H, $PhCH_2$), 4.66 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.59-4.50 (m, 5H, $PhCH_2$), 4.46 (d, 1H, J = 7.5 Hz, H-1′), 4.37 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_2$), 4.29-4.23 (m, 2H, H-1, $PhCH_2$), 4.18 (q, 1H, J = 6.6 Hz, H-5″), 4.13 (ddd, 1H, H-5′), 4.06 (dd, 1H, J = 10.2, 3.0 Hz, H-2″), 4.00 (d, 1H, J = 3.0 Hz, H-4′), 3.97-3.93 (m, 2H, H-3″, H-4), 3.84 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5,6}$ = 4.2 Hz, H-6a), 3.75 (dd, 1H, J_{gem} = 10.8, $J_{5,6}$ = 1.2 Hz, H-6b), 3.60-3.55 (6H, H-2′, H-3, H-4″, OCH_3), 3.49 (dd, 1H, J_{gem} =

9.6 Hz, $J_{5',6'}$= 5.4 Hz, H-6'a), 3.39-3.35 (m, 2H, H-2, H-5), 3.29-3.25 (m, 2H, H-3, H-6'b), 0.89 (d, 3H, J = 6.6 Hz, H-6″). ^{13}C NMR (125 MHz) δ 139.1, 138.8, 138.7, 138.6, 138.5, 138.4, 104.6, 102.9, 100.2, 82.4, 81.7, 81.2, 79.6, 79.0, 78.0, 76.5, 75.1, 75.0, 74.9, 74.8, 74.7, 74.4, 73.6, 73.3, 73.0, 72.7, 72.0, 68.3, 67.7, 67.0, 66.5, 57.0, 35.5, 16.4. ES HRMS Calcd. for $C_{82}H_{85}O_{15}Na$ (M+Na):1335.6015. Found 1335.5999.



Methyl α-D-fucopyranosyl-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-gluco-pyranoside (26).

The protected trisaccharide **96** (66mg, 50.3 umol) was dissolved in methanol (5 ml). Palladium hydroxide (15 mg) under argon was added and the mixture was stirred for 4 h. The solution was filtered and purified with a Waters C-18 Sep-Pak solid-phase cartridge (0-50 % MeOH). The product was collected and lyophilized to give a white powder (20 mg, 80 %): $[\alpha]_D$ +72.8 (c = 0.1, H_2O). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.86 (d, 1H, J = 4.0 Hz, H-1″), 4.50-4.45 (m, 2H, H-1′, H-5″), 4.40 (d, 1H, J = 8.0 Hz, H-1), 4.01-3.97 (m, 2H, H-4′, H-5), 3.93-3.88 (m, 2H, H-3″, H-6′a), 3.84-3.80 (m, 3H, H-4″, H-6a, H-6′b), 3.79-3.73 (m, 3H, H-2″, H-3′, H-5′), 3.66-3.62 (m, 2H, H-3, H-6b), 3.61-3.54 (m, 5H, H-2′, H-4, OCH_3), 3.31-3.28 (m, 1H, H-2), 1.17 (d, 3H, J = 6.5 Hz, H-6″). ^{13}C NMR (125 MHz) δ 104.1, 103.9, 101.0, 79.5, 77.8, 76.3, 75.7,

75.3, 73.7, 72.8, 72.7, 71.6, 70.2, 69.2, 68.0, 61.1, 60.9, 58.0, 16.1. ES HRMS Calcd. for $C_{19}H_{34}O_{15}Na$ (M+Na):525.1789. Found 525.1789.

5.1.4 FT-ICR Mass Assay

All experiments were performed on an Apex II Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker, Billerica, MA) equipped with an external nanoES ion source. NanoES was performed using an aluminosilicate capillary (1.0 mm o.d., 0.68 mm i.d.), pulled to ~4 – 7 μm o.d. at one end using a P-2000 micropipette puller (Sutter Instruments, Novato, CA). The electric field required to spray the solution was established by applying a voltage of ~800 V to a platinum wire inserted inside the glass tip. The solution flow rate was typically 20 - 50 nL/min. The droplets and gaseous ions produced by nanoES were introduced into the mass spectrometer through a stainless steel capillary (i.d. 0.43 mm) maintained at an external temperature of 66 °C. The ion/gas jet sampled by the capillary (48 - 52V) was transmitted through a skimmer (0 - 2V) and stored electrodynamically in an rf hexapole. A hexapole accumulation time of between 1.5 and 2.0 s was used for all experiments. Ions were ejected from the hexapole and accelerated to ~-2700V into a 9.4 T superconducting magnet, decelerated, and introduced into the ion cell. The trapping plates of the cell were maintained at a constant potential of 1.4 - 1.8 V throughout the experiment. The typical base pressure for the instrument was ~$5x10^{-10}$ mbar.

Data acquisition was controlled by an SGI R5000 computer running the Bruker Daltonics XMASS software, version 5.0. Mass spectra were obtained using standard

experimental sequences with chirp broadband excitation. The time domain signal, consisting of the sum of 20 - 40 transients containing 128 K data points per transient, were subjected to one zero-fill prior to Fourier transformation.

Chapter 6

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